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# MUTAGENICITY OF SOME MUNITION WASTEWATER CHEMICALS AND CHLORINE TEST KIT REAGENTS

*Final Report*

VINCENT F. SIMMON, RONALD J. SPANGGORD,
SHARON ECKFORD, and VERNON McCLURG

*May 1977*

*Supported by*

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Dr. William F. Cowen, Project Officer
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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assemble, and pack (LAP) wastewater: 7-50 LAP, 7-100 LAP, 9-100 LAP; condensate water, N,N-diethyl-p-phenylenediamine oxalate (DPO), N,N-dimethyl-p-phenylenediamine sulfate (DPS), syringaldazine, mutagenicity assays, Salmonella typhimurium, Saccharomyces cerevisiae.

20 ABSTRACT (Continued)

Before the application of disinfection techniques, 1,3,5-trinitrobenzene, pH7-50% photolyzed LAP (Load, Assemble, and Pack plant wastewater), pH7-100% photolyzed LAP, pH9-100% photolyzed LAP, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde, N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate were found to have mutagenic activity at their aqueous solubility concentrations. 1,3-Dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, trinitroglycerine, 2,4,6-trinitrotoluene, RDX, condensate water from TNT manufacture, syringaldazine, 2,4,6-trinitroresorcinol, PETN, and HMX did not show mutagenicity before chlorination or ozonation.

Under the chlorination conditions used, only five compounds (2,4,6-trinitroresorcinol, N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, syringaldazine, and 2,4,6-trinitrobenzaldehyde) underwent significant reaction. 2,4,6-Trinitroresorcinol and syringaldazine remained nonmutagenic after chlorination. The mutagenic activity of N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, and 2,4,6-trinitrobenzaldehyde increased slightly after chlorination.

Under the ozonation conditions, seven compounds (1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene, 2,6-dinitrotoluene, 1,3-dinitrobenzene, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitroresorcinol, and 7-50 LAP) underwent significant reaction. Of these, only 1,3,5-trinitrobenzene, 7-50 LAP, and 2,4,6-trinitrobenzaldehyde were mutagenic before ozonation. 2,4,6-Trinitrobenzaldehyde was the only compound that appeared to have a slight increase in mutagenic activity as the result of ozonation. We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments. However, the concentrations tested were very low (upper limit was water solubility) and the possibility of false negative results should be considered.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds

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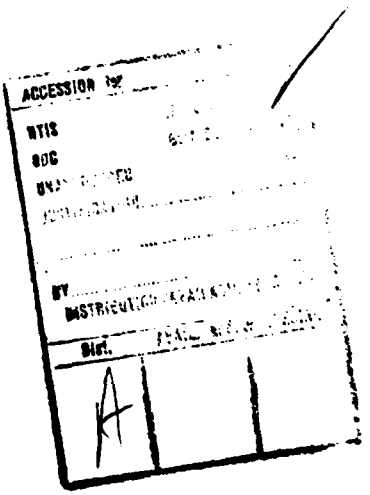
gave a negative response because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.



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## EXECUTIVE SUMMARY

In this study, 20 compounds including munition wastewater chemicals, photolyzed wastewaters, and chlorine test kit reagents were evaluated for mutagenic activity at aqueous solubility levels before and after application of ozone or chlorine disinfection techniques.

Before the application of disinfection techniques, 1,3,5-trinitrobenzene, pH7-50% photolyzed LAP (Load, Assemble, and Pack plant wastewater), pH7-100% photolyzed LAP, pH9-100% photolyzed LAP, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde, N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate were found to have mutagenic activity at their aqueous solubility concentrations. 1,3-Dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, trinitroglycerine, 2,4,6-trinitrotoluene, RDX, condensate water from TNT manufacture, syringaldazine, 2,4,6-trinitroresorcinol, PETN, and HMX did not show mutagenicity before chlorination or ozonation.

Under the chlorination conditions used, only five compounds (2,4,6-trinitroresorcinol, N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethl-p-phenylenediamine sulfate, syringaldazine, and 2,4,6-trinitrobenzaldehyde) underwent significant reaction. 2,4,6-Trinitroresorcinol and syringaldazine remained nonmutagenic after chlorination. The mutagenic activity of N,N-diethyl-p-phenylenediamine oxalate, N,N-dimethyl-p-phenylenediamine sulfate, and 2,4,6-trintrobenzaldehyde increased slightly after chlorination.

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We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments. However, the concentrations tested were very low (upper limit was water solubility) and the possibility of false negative results should be considered.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds gave a negative response because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.

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# INTRODUCTION

The use of chlorine in water purification as a disinfectant and oxidant has come under critical review in light of the findings of low levels of chlorinated organics in drinking water.[1] These newly formed organics may be detrimental to both man and the environment and should be investigated relative to their biological activity and potential health hazard.

The potential for formation of hazardous products is not unique to chlorine treatment of water since the potential exists with any treatment in which chemical transformation of organics is likely to occur. Therefore, purification methods in which ozone, chlorine dioxide, and halogens are used are subject to the same problems that are encountered with the use of chlorine as a disinfectant.

In this study, we evaluated the effects of ozonation or chlorination of 13 military-unique wastewater chemicals, 4 wastewater mixtures, and 3 reagents for chlorine test kits. The military chemicals tested were 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, trinitroglycerine, 2,4,6-trinitrobenzaldehyde, pentaerythritol tetranitrate (PETN), 2,4,6-trinitrobenzonitrile, 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX), 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX), 2,6-dinitrotluene, 2,4-dinitrotoluene, 3,5-dinitrotoluene, 2,4,6-trinitrotoluene (TNT), and 2,4,6-trinitrorescorcinol.

The wastewater tested was load, assemble, and pack (LAP) wastewater obtained from Jolliet Army Ammunition Plant (JAAP). This wastewater was adjusted to pH 7 and photolytically degraded (through Pyrex filters) in a laboratory reactor (Figure 1) to reduce the TNT concentration to 50% (7-50 LAP) and 100% (7-100 LAP) of the initial value. Another sample was adjusted to pH 9, and the TNT was 100% photolytically decomposed (9-100 LAP). Condensate water, which arises from the evaporative treatment of Sellite process waters at TNT production facilities, was obtained from JAAP and tested directly.

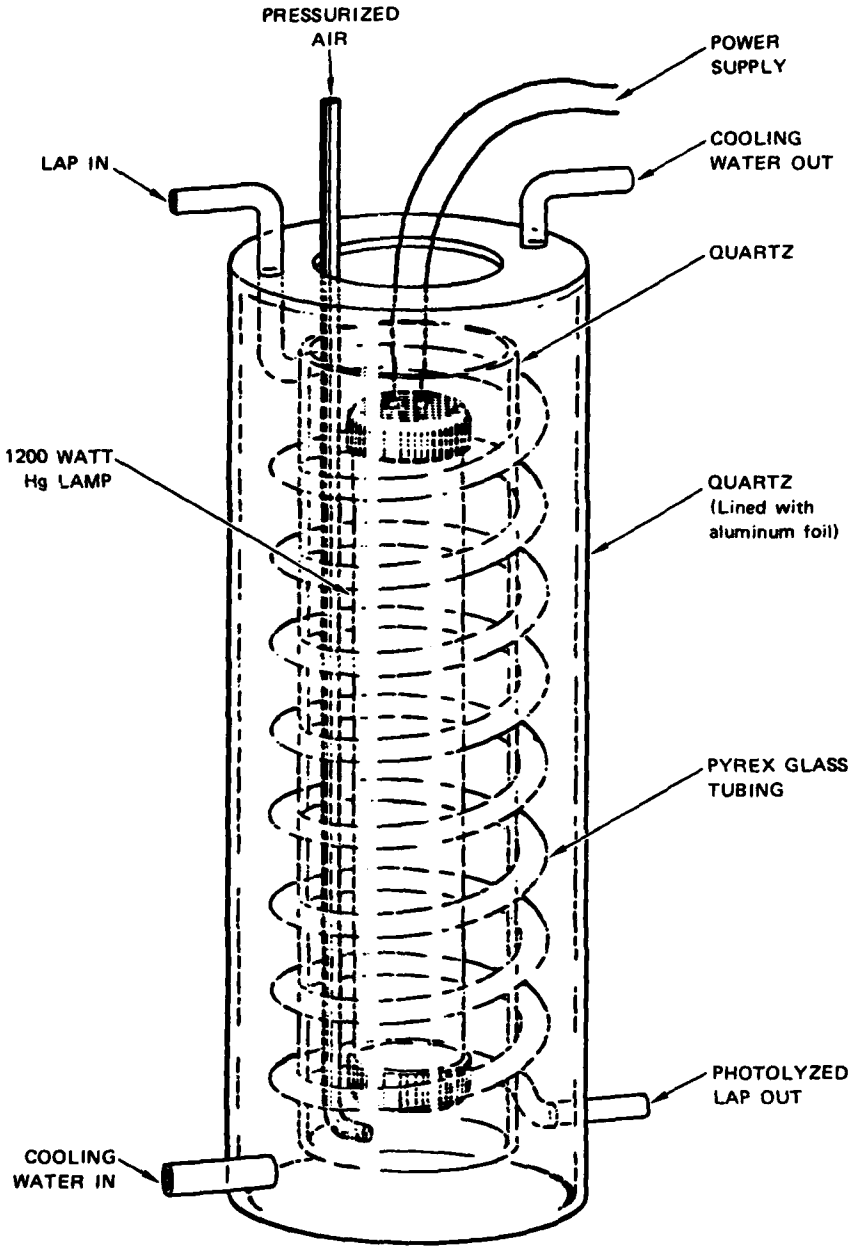


FIGURE 1 DIAGRAM OF FLOW-THROUGH PHOTOLYTIC REACTOR

The chlorine test reagent chemicals tested were N,N-diethyl-p-phenylenediamine oxalate (DPO) and syringaldazine. N,N-Dimethyl-p-phenylenediamine sulfate (DPS) was also supplied. It differs from DPO in counter-ion and has N-methyl groups instead of N-ethyl groups, but it is not used in chlorine test kits; it was tested for comparative results. DPO is the same chemical as DPD (diethylphenylenediamine), DPD being the more common abbreviation for this reagent, which is used for chlorine determinations. Syringaldazine is being considered as a substitute for DPD[2] in chlorine determinations.

One possible way of evaluating the safety of a particular water purification technique is through the use of rapid microbial bioassays, which serve as a prescreening method for assessing the mutagenic activity of organics resulting from the purification technique. The biological assays used to determine whether ozonation or chlorination produced biologically active (i.e., mutagenic) products were the Ames Salmonella/microsome assay (reversion to histidine independence in five strains of Salmonella typhimurium) and mitotic recombination in the yeast Saccharomyces cerevisiae D3. A rat-liver postmitochondrial supernatant fraction was incorporated into the assay procedure to provide mammalian metabolic pathways.

The objective of this project was to evaluate the mutagenic activity of compounds produced by the reaction of chlorine or ozone with selected military-unique compounds. This effort is to assess potential health hazards resulting from discharges of pollutants into surface waters where ozone or chlorine may be used for water treatment before distribution from a water municipality.

To achieve this objective, the test chemicals were screened for mutagenic activity before ozone or chlorine treatment in water and then were evaluated for their reactivity with each reagent. The treated test chemical solutions were evaluated for enhanced mutagenicity relative to untreated test chemical solutions. Products formed were chemically evaluated to identify the products that might be responsible for increased mutagenic activity.

Preliminary results of this research were presented to the Society of Toxicology at its annual meeting in March, 1977. The abstract of this presentation is presented in Appendix C.

## METHODS

### Chlorination

Solutions of hypochlorous acid (HOCl) were prepared by acidification of calcium hypochlorite (Fisher Chemical Co.) with hydrochloric acid in deionized water and buffered to pH 7 with commercial phosphate buffer (Micro Essential Laboratory). Initial stock chlorine concentrations were determined by standard iodometric titration.[3] Final chlorine concentrations after reaction with the test chemical were adjusted to 0.1 to 0.2 mg/1 with sodium thiosulfate and determined by the syringaldazine method[4] before mutagenic screening.

Chlorinations were performed in Pyrex Erlenmeyer flasks that had been stoppered and covered with aluminum foil. Each solution was stirred with the aid of a Teflon-coated magnetic stir-bar throughout the contact time, which ranged from 30 minutes to more than 48 hour.

### Ozonation

Ozonations were performed in an all-Pyrex glass reactor, pictured in Figure 2, equipped with an overhead stainless-steel stirrer. Ozone, generated from a Welsback Model T-408 ozone generator, was bubbled through the reactor so that 4 mg/1 of ozone was introduced into the system. Aqueous solutions of the test compound in deionized water were buffered with phosphate at pH 7, and ozone contract times of 10 minutes were used. Ozone concentrations were monitored by standard iodometric titrations.[3]

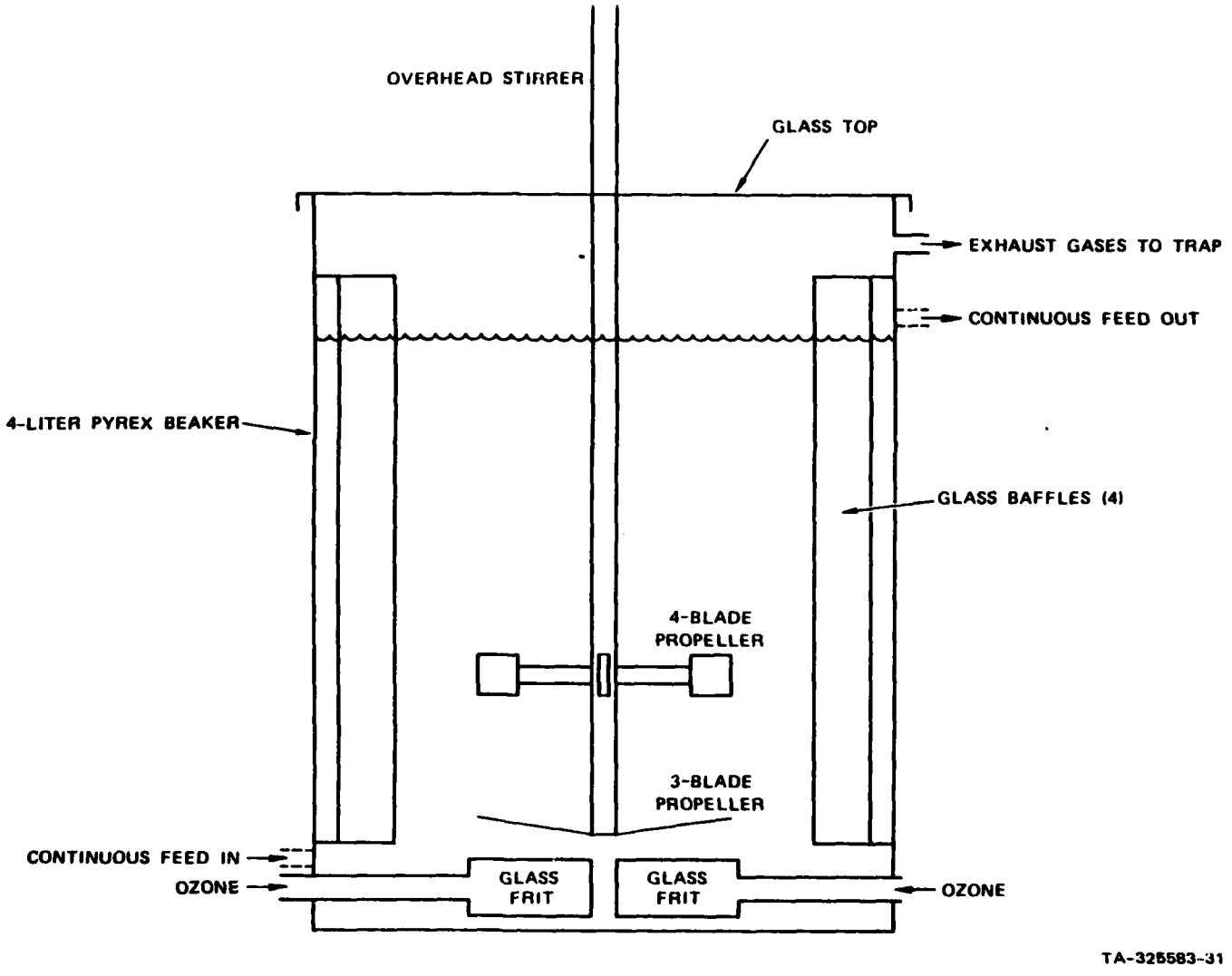


FIGURE 2 SCHEMATIC DIAGRAM OF OZONATION REACTION VESSEL

## Microbiological Mutagenicity Assays

### Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The his$^+$ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.[5-9] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa$^-$) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of vitamin biotin (bio$^-$) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB$^-$). The rfa$^-$ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrB$^-$ mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations

for a given dose of most mutagens.[9] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. We have shown that TA100 can detect mutagens, such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), that are not detected by strain TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens (e.g., ICR-191, benzo(a)pyrene, aflatoxin $B_1$, and 7,12-dimethylbenz(a)anthracene). Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthracenes, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All the indicator strains are routinely checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. Cultures are then stored in 10% sterile glycerol at -80°C. For each experiment, an inoculum from the stock cultures is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals, either of the aromatic amino type or polycyclic hydrocarbon type, are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[8,10-12] Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.[10] In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of $H_2O$.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)
(4) Up to 0.25 ml of a solution of the test chemical.

For negative controls, we use steps (1), (2), and (3) (optional). For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3) (optional), and (4).

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* 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.

This mixture is stirred gently and then poured onto minimal agar plates.* After the top agar has set, the plates are incubated at 37° C for 2 days. The number of his$^+$ revertant colonies is counted and recorded.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.[13] When grown on a medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.[14]

The S. cerevisiae tester strain is stored at -80° C. For each experiment, the tester strain in inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 37° C with aeration.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged, and the cells are resuspended at a concentration of $10^8$ cells/ml in a .067 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.0 ml of the organisms
- 0.5 ml of either the metabolic activation mixture or buffer
- 1.0 ml of the test chemical.

Several doses of the chemical (up to 5%, w/v or v/v, or maximum water solubility) are tested in each experiment, and appropriate controls are included.

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* Minimal agar plates consist of, per liter, 15 g of agar, 50 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of $K_2HPO_4$, and 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$.

The suspension mixture is incubated at 30° C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline, and a volume of 0.2 ml of the $10^{-5}$ and $10^{-3}$ dilutions is spread on tryptone-yeast agar plates; five plates are used for the $10^{-3}$ dilution and three plates are used for the $10^{-5}$ dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the $10^{-3}$ dilution are scanned with a dissecting microscope at 10 X magnification, and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the $10^{-5}$ dilution.

The number of mitotic recombinants is calculated per $10^5$ survivors. A positive response in this assay is indicated by a dose-related increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per $10^5$ survivors.

## RESULTS AND DISCUSSION

### Chlorination

The results for the chemicals subjected to chlorine water-treatment facility conditions (10 ppm chlorine, pH 7, 0.5 hr contact time) are given in Table 1. The only compounds that were found to undergo significant reaction were 2,4,6-trinitroresorcinol, DPO, DPS, syringaldazine, and 2.4,6-trinitrobenzaldehyde.

The recalcitrant nature of the majority of the compounds studied under these chlorination conditions is not surprising in light of the known pathways of chlorine transformation of organics, including substitution, addition, and oxidation reactions.[15] Photochemical processes were eliminated by performing the reactions in flasks covered with aluminum foil.

Of the above pathways, oxidative transformation appeared to be the most probable mode of decomposition for the munition compounds, and attempts were made to increase rates of transformation by varying chlorine concentrations, pH, temperature, and contact time. It was hoped that stressed conditions would produce significant concentration levels of degradation products for mutagenic screening.

Using TNT as a prototype compound to study reaction conditions, a 100-ppm solution of TNT at pH 7 was allowed to stir with 1000 ppm of hypochlorous acid. Samples were analyzed for TNT at intervals of 1, 2, and 24 hours, and essentially all the starting material was recovered. In a similar study performed at pH of 2, 4, 7, and 8 (variable hypochlorous acid concentrations), no TNT decomposition was noted over a 24-hour period. Finally, temperatures were varied, and TNT decomposition occurred at 75° C and above.

Table 1

REACTIVITY OF TEST MATERIALS SUBJECTED TO 10 ppm CHLORINE FOR 30 MINUTES AT pH 7 (Buffered with phosphate)

| Compound | Initial Concentration (ppm) | Percentage Reacted |
|---|---|---|
| 3,5-Dinitrotoluene | 127 | 0 |
| 2,4,6-Trinitroresorcinol | 518 | 4.8 |
| 2,4,6-Trinitrotoluene | 139 | 0 |
| 2,4-Dinitrotoluene | 212 | 0 |
| Condensate water | 67* | 0 |
| N,N-Diethyl-p-phenylenediamine oxalate | 226 | 20 |
| N,N-Dimethyl-p-phenylenediamine sulfate | 139 | 27 |
| Syringaldazine | 122 | 34 |
| 7-50 LAP | 17† | 0 |
| RDX | 47 | 0 |
| HMX | 1 | 0 |
| 2,6-Dinitrotoluene | 179 | 0 |
| Pentaerythritol tetranitrate | 1 | 0 |
| 1,3,5-Trinitrobenzene | 117 | 0 |
| 2,4,6-Trinitrobenzaldehyde | 59 | 25 |
| Trinitroglycerine | 800 | 0 |
| 1,3-Dintrotoluene | 126 | 0 |
| 1,3-Dinitrobenzene | 126 | 0 |

* Based on 2,4-dinitrotoluene concentration.
† Based on 2,4,6-trinitrotoluene concentration.
‡ -- No parameter used to measure reactivity.

A second series of experiments was designed to detect mutagenicity under stressed chlorination conditions. The pH of a 1000-ppm solution of chlorine was adjusted to 4 with hydrochloric acid (no buffer), and the solution was allowed to react with the munition compound for 2 hours at 70 to 80° after standing at room temperature for 48 hours. Under these conditions, slight reactions of the munition compounds were observed. These data are summarized in Table 2. No reaction products were observed in either high-pressure liquid chromatographic (hplc) or gas chromatographic (gc) profiles of these solutions.

Ozonation

The results for chemicals subjected to ozone treatment are presented in Table 3. The nitroaromatics react readily with ozone in aqueous solution. The "Percentage Reacted" in Table 3 should not be construed as an order of reactivity since in each case ozone is the limiting reagent and it is possible that intermediate products (such as nitrobenzaldehydes from the nitrotoluenes) consume ozone at a faster rate than does the parent compound. To better understand reactivity, the extent of reaction was calculated by first computing the maximum percentage of reaction (defined as the moles of ozone divided by the moles of substrate) and dividing this value into the observed "Percentage Reacted" as shown below:

Moles ozone/moles substrate = maximum % of reaction

$$\frac{\text{\% reacted}}{\text{max \% of reaction}} = \text{extent of reaction.}$$

The extent of reaction values can be used to predict reactivity between structurally related compounds (such as related nitrotoluenes and related nitrobenzenes) and indicate autocatalytic effects or the participation of oxygen in the oxidation reactions (2,4,6-trinitroresorcinol).

Several compounds yielded products that were identified by gas chromatography/mass spectroscopy (gc/ms). From TNT, trinitrobenzene was obtained; this suggests alkyl oxidation rather than electrophilic substitution on the deactivated aromatic ring (Eq. 1). Alkyl oxidation

Table 2

AQUEOUS CHLORINATIONS OF SELECTED TEST MATERIALS: 1000 ppm $Cl_2$ AT VARIOUS TIME INTERVALS

| Compound | Initial Concentration (ppm) | Contact Time and Temperature | Percentage Reacted | pH | |
|---|---|---|---|---|---|
| | | | | Initial | Final |
| 3,5-Dinitrotoluene | 72 | 2 hr, 70° | 2 | 4.0 | 4.6 |
| 2,4,6-Trinitrotoluene | 106 | 2 hr, 70° | 3 | 4.0 | 5.3 |
| 2,4-Dinitrotoluene | 138 | 2 hr, 70° | 5 | 4.0 | 5.3 |
| 1,3-Dinitrobenzene | 251 | 2 hr, 70° | 0 | 4.0 | 3.5 |
| RDX | 20 | 2 hr, 70° | 0 | 4.0 | 3.6 |
| 7-50 LAP | 21.3* | 2 hr, 70° | 33 | 4.0 | 3.0 |
| Condensate water | 51 | 2 hr, 70° | 12 | 4.0 | 3.3 |
| HMX | 3 | 2 hr, 70° | 8 | 4.0 | 4.1 |
| 2,4,6-Trinitroresorcinol | 160 | 2 hr, rt | 91 | 4.0 | 3.0 |
| N,N-Diethyl-p-phenylenediamine oxalate | 4377 | 2 hr, 70° | 100 | 4.0 | 2.2 |
| N,N-Dimethyl-p-phenylenediamine sulfate | 2195 | 2 hr, 70° | 100 | 4.0 | 2.3 |

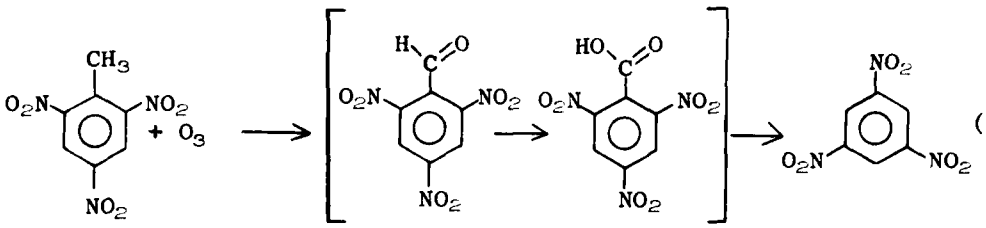
* Based on 2,4,6-trinitrotoluene concentration.

Table 3

REACTIVITY OF MUNITION MATERIALS WHEN SUBJECTED TO 4 mg/l OZONE FOR 30 MINUTES AT BUFFERED pH 7

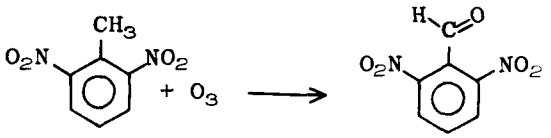
| Compound | Initial Concentration (ppm) | Percentage Reacted | Max. Reaction (%) | Extent of Reaction (%) |
|---|---|---|---|---|
| Trinitrobenzene | 362 | 2.4 | 4.0 | 60 |
| 2,4,6-Trinitrotoluene | 30 | 15.5 | 59 | 26 |
| 2,6-Dinitrotoluene | 139 | 20 | 27 | 74 |
| 1,3-Dinitrobenzene | 52 | 25 | 26 | 96 |
| 2,4,6-Trinitroresorcinol | 308 | 12 | 5.4 | 225 |
| 2,4,6-Trinitrobenzaldehyde | 71 | 11 | 28 | 39 |
| 7-50 LAP | 32* | 3 | 59 | 5 |
| Pentaerythritol tetranitrate | 1 | 0 | 0 | 0 |
| 2,4,6-Trinitrobenzonitrile | ND† | NT‡ | NT | NT |
| 7-100 LAP | 0* | NT<br>NA§ | NT<br>NA | NT<br>NA |

* Based on 2,4,6-TNT concentration.
† ND, not determined; chemical hydrolyses in water.
‡ NT, not tested.
§ NA, not applicable because of complete photolysis of 2,4,6-TNT in 7-100 LAP.



(Eq. 1)

also occurred with 2,6-dinitrotoluene, and 2,6-dinitrobenzaldehyde was identified as a reaction product (Eq. 2).



(Eq. 2)

In general, very few products resulting from ozonation of the munitions compounds possessed sufficient volatility for gc/ms analysis or sufficient absorbance at 254 nm for detection in hplc profiles. These phenomena would be expected when the aromatic ring is ruptured.

Microbiological Mutagenicity

All 20 compounds were subjected to chlorine water treatment facility conditions (10 ppm chlorine, pH 7, 0.5 hr contract time). Also, 15 of the compounds were chlorinated with 1000 ppm chlorine, pH 4, 48 hr contact time at 85° C. Mutagenic assays of the compounds showed no significant differences between the two chlorination processes. The concentrations for each chemical are listed in Tables 1 and 2.

Table 4 summarizes the results of mutagenic screening tests on the 20 compounds. It should be noted that, in general, the concentrations tested were low. This was necessitated by the requirement that the compound be dissolved in water. Usually, dimethylsulfoxide (DMSO) would be used to achieve higher concentrations for testing. Thus, the absence of mutagenic activity in these assays should not be construed as proof that a compound is not mutagenic. Rather, it can only be stated that at the highest concentrations tested, the compound was not mutagenic. (1,3-Dinitrobenzene, 2,4-dinitrotoluene,

2,6-dinitrotoluene, 3,5-dinitrotoluene, and 2,4,6-trinitrotoluene have been found to be mutagenic when tested at doses higher than those reported in this study.[16]

Moreover, certain classes of carcinogens are not detected as mutagens by the Salmonella/microsome procedure (e.g., inorganics, hormones, polychlorinated hydrocarbons such as chloroform, carbon tetrachloride, dieldrin, and DDT).

1,3,5-Trinitrobenzene was mutagenic on S. typhimurium strains TA98 and TA100 before and after ozonation and chlorination, with and without metabolic activation. The mutagenic activity was not significantly altered by disinfection treatment. In most cases, there was much less mutagenic activity with the metabolic activation system. Trinitrobenzene was not mutagenic on S. cerevisiae. (Tables A-1 through A-5 and B-1 through B-4).

1,3-Dinitrobenzene was not mutagenic in these assays before or after chlorination or ozonation (Tables A-6 through A-11 and B-5 through B-8).

Trinitroglycerine was not mutagenic before or after chlorination in these assays (Tables A-12 through A-14 and B-9 and B-10).

Pentaerythritol tetranitrate (PETN) was not mutagenic on S. typhimurium or S. cerevisiae before or after chlorination or ozonation (Tables A-15 to A-18 and B-11 through B-14).

Condensate water was not mutagenic on S. typhimurium or S. cerevisiae before or after chlorination (Tables A-19 and A-20 and B-15 and B-16).

Syringaldazine was not mutagenic on S. typhimurium and S. cerevisiae before or after chlorination or ozonation (Tables A-21 and A-22 and B-17 and B-18).

HMX and RDX were not mutagenic before or after chlorination in these assays (Tables A-23 through A-28 and B-19 through B-22).

Table 4

IN VITRO MICROBIOLOGICAL ASSAYS WITH MUNITION WASTEWATER CHEMICALS AND CHLORINE TEST KIT REAGENTS

Absence of Mutagenic Activity (-); Presence of Mutagenic Activity (+)

| Compound | Salmonella typhimurium | | | | Saccharomyces cerevisiae | | | | Reactivity | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Chlorination | | Ozonation | | Chlorination | | Ozonation | | | |
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Chlorination | Ozonation |
| 1,3,5-Trinitrobenzene | + | + | + | + | - | - | - | - | - | + |
| 1,3-Dinitrobenzene | - | - | - | - | - | - | - | - | - | + |
| Trinitroglycerine | - | - | | | - | - | | | - | NT* |
| Pentaerythritol tetranitrate | - | - | - | - | - | - | - | - | - | - |
| Condensate water | - | - | | | - | - | | | - | NT |
| Syringaldazine | - | - | | | - | - | | | + | NT |
| HMX | - | - | | | - | - | | | - | NT |
| RDX | - | - | | | - | - | | | - | NT |
| N,N-Diethyl-p-phenylenediamine oxalate | +w/ma | + | | | - | - | | | + | NT |
| N,N-Dimethyl-p-phenylenediamine sulfate | + | + | | | - | - | | | + | NT |
| 7-50 LAP | + | ++† | + | + | - | - | - | - | ND* | + |
| 7-100 LAP | + | +↓‡ | + | + | - | - | - | - | ND | ND |
| 2,6-Dinitrotoluene | - | - | - | - | - | - | - | - | - | + |
| 2,4-Dinitrotoluene | - | - | | | - | - | | | - | NT |
| 3,5-Dinitrotoluene | - | - | | | - | - | | | - | NT |
| 2,4,6-Trinitrotoluene | - | - | - | - | - | - | - | - | - | + |
| 2,4,6-Trinitroresorcinol | - | - | - | - | - | - | - | - | + | + |
| 2,4,6-Trinitrobenzonitrile | + | + | + | + | - | - | - | - | - | ND |
| 2,4,6-Trinitrobenzaldehyde | + | ++ | + | + | - | - | - | - | + | + |

* ND, not determinable; NT, not tested.

† ↑Post-chlorination samples show an increase in mutagenic activity over the pre-chlorination samples.

‡ ↓Post-chlorination samples show a decrease in mutagenic activity over the pre-chlorination samples.

DPO and DPS were mutagenic before and after chlorination. However, chlorination markedly decreased the mutagenicity of DPS. With both compounds, metabolic activation was required for mutagenicity, although, in some instances, DPS appeared to be weakly mutagenic without metabolic activation.

Both DPO and DPS form purple solutions when they are added to water, and the color darkens with time. Although the data are not presented, we observed that solution of freshly prepared DPS was less mutagenic if the distilled water in which it was prepared was bubbled with nitrogen (presumably this would remove oxygen). After 1 day of standing at room temperature, the mutagenic activity of both nitrogen-treated and untreated DPS had increased approximately 10-fold.

Of the two compounds, DPS was the more mutagenic (approximately 3-fold). Mutagenic activity was observed primarily in strains TA1538 and TA98. DPO was weakly mutagenic in TA100 and DPS was weakly mutagenic in TA1537 and TA100.

Our results suggest that both compounds are frameshift mutagens. Preliminary results suggest that the mutagenic activity may be attributable to some oxidation product(s) of these compounds. It seems likely that the difference in mutagenic activity between the two compounds is due to the methyl and ethyl groups rather than to the salts (oxalate and sulfate). Tables A-29 through A-34 and B-23 through B-28 present the results from assays on DPO and DPS.

7-50 LAP was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 before and after chlorination and ozonation. Somewhat more mutagenic activity was observed after chlorination than before treatment. This compound was more mutagenic without metabolic activation than with metabolic activation. No mutagenicity was observed with S. cerevisiae (Tables A-35 through A-40 and B-29 through B-33).

7-100 LAP, like 7-50 LAP, was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 before and after chlorination and ozonation, particularly without metabolic activation. Unlike 7-50 LAP,

there was a slight reduction in mutagenic activity after chlorination. No mutagenicity was observed with S. cerevisiae (Tables A-41 through A-45 and B-34 through B-37).

9-100 LAP was mutagenic on S. typhimurium strains TA1538, TA98, and TA100 before and after chlorination. There was somewhat more activity without metabolic activation. 9-100 LAP was not mutagenic in assays with S. cerevisiae (Tables A-46 and A-47 and B-38).

2,6-Dinitrotoluene was tested twice on S. typhimurium before and after chlorination. In one test at 2.4 ppm, it showed slight mutagenicity after chlorination; in a second test at 58.7 ppm, it showed a mutagenic dose response after chlorination. It was not mutagenic on S. typhimurium either before or after ozonation, nor was it mutagenic on S. cerevisiae either before or after chlorination or ozonation (Tables A-48 through A-52 and B-39 through B-42).

2,4-Dinitrotoluene was not mutagenic either before or after chlorination, nor was it mutagenic in any of the S. cerevisiae assays (Tables A-53 through A-55 and B-43 and B-44).

3,5-Dinitrotoluene was not mutagenic either before or after chlorination on S. typhimurium or on S. cerevisiae (Tables A-56 and A-57 and B-45 and B-46).

2,4,6-Trinitrotoluene was not mutagenic either before or after chlorination or ozonation in any of the assays performed. In two experiments (#18 and 28), it appeared to be weakly mutagenic (< 2-fold increase in revertants) on TA100 without metabolic activation (Tables A-58 through A-62 and B-57 through B-50).

2,4,6-Trinitroresorcinol was not mutagenic on S. typhimurium either before or after ozonation or chlorination, nor was it mutagenic in any of the S. cerevisiae assays (Tables A-63 through A-67 and B-51 through B-54).

2,4,6-Trinitrobenzonitrile was mutagenic on S. typhimurium strains TA98 and TA100 before and after ozonation and chlorination. In general, it was slightly less mutagenic after chlorination than before, and it

was more than twice as mutagenic after ozonation than before. Metabolic activation was not required for mutagenicity. Trinitrobenzonitrile was not mutagenic on S. cerevisiae before or after ozonation or chlorination (Tables A-68 through A-73 and B-55 through B-58).

2,4,6-Trinitrobenzaldehyde was mutagenic on S. typhimurium strains TA1537, TA1538, TA98, and TA100 both before and after chlorination and ozonation. Neither treatment appeared to significantly alter the mutagenic response, although significant (5 to 30%) chemical reaction appears to have occurred. Metabolic activation decreased mutagenicity. This compound was not mutagenic in any of the assays with S. cerevisiae. Tables A-74 through A-80 and B-59 through B-62 present the results.

Amino-dinitrotoluenes. Additionally, we investigated three amino-dinitrotoluenes as potentially reactive components in condensate water. The compounds (5-amino-2,4-dinitrotoluene; 4-amino-3,5-dinitrotoluene; and 4-amino-2,6-dinitrotoluene) were allowed to react with chlorine under the conditions previously described. As shown in Table 5, the two 4-amino compounds showed significant reactivity (75%) with chlorine, but the reactivity was only 12% for the 5-amino compound. No new products were evident in gc profiles of the reacted solutions. Also, there was no indication of mutagenic activity associated with the reacted solutions.

Table 5

EFFECT OF CHLORINATION ON AMINO-DINITROTOLUENES

| Compound | Initial Concentration (ppm) | Percentage Reacted | Mutagenicity* | | | |
|---|---|---|---|---|---|---|
| | | | Pre | | Post | |
| | | | Bacteria | Yeast | Bacteria | Yeast |
| 5-Amino-2,4-dinitrotoluene | 3 | 12 | -- | -- | -- | -- |
| 4-Amino-3,5-dinitrotoluene | 18 | 68 | -- | -- | -- | -- |
| 4-Amino-2,6-dinitrotolene | 20 | 81 | -- | -- | -- | -- |

* -- No indication of mutagenic activity.

## CONCLUSIONS

Eight of the compounds assayed were mutagenic at aqueous solubility before disinfection treatment. The mutagenic compounds were 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzonitrile, 2,4,6-trinitrobenzaldehyde (munition wastewater chemicals), 7-50 LAP, 7-100 LAP, and 9-100 LAP (photolyzed wastewaters), N,N-diethyl-p-phenylenediamine oxalate, and N,N-dimethyl-p-phenylenediamine sulfate (chlorine test kit reagents).

In other studies we have conducted under contract to the U.S. Army Bioengineering Research and Development Laboratory, we have found that 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 3,5-dinitrotoluene, and 2,4,6-trinitrotoluene are mutagenic in the Salmonella/microsome assay when tested at higher doses. We are not aware of mutagenicity assays at higher doses on other munition wastewater chemicals (e.g., trinitroglycerine, pentaerythritol tetranitrate, HMX, RDX, etc.).

The results of these experiments suggest that although the nitroaromatics react readily with ozone, any mutagenic activity that they showed was not greatly atlered by ozonation. Only a few of the compounds reacted with chlorine. Based on these experiments, it would appear that disinfection treatment does not greatly alter mutagenic activity. For compounds that were mutagenic before treatment, both slight increases and slight decreases in mutagenic activity were observed after treatment. We did not observe any cases in which nonmutagenic compounds were converted into mutagens by either of the disinfection treatments.

None of the chemicals tested was mutagenic in assays with S. cerevisiae D3 under the assay conditions used in these experiments. It is possible that these compounds gave a negative response

because they are not strongly mutagenic in this procedure, particularly in view of the relatively low concentrations of test compounds. Alternatively, these compounds may not be mutagens in S. cerevisiae D3 mitotic recombination assay. We conclude that this indicator microorganism is not useful in evaluating the mutagenicity of these compounds.

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# APPENDIX A

## IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

## APPENDIX A--*IN VITRO* ASSAYS WITH *SALMONELLA TYPHIMURIUM*

Table A-1

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 5

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 67 | 11 | 22 | 32 | 92 |
| | + | | 102 | 13 | 16 | 28 | 114 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 157 | | | | |
| 9-Aminoacridine | - | 100 | | 1252 | | | |
| 2-Nitrofluorene | - | 50 | | | 9 | | |
| AF2 | - | 0.1 | | | | 218 | 554 |
| 2-Anthramine | + | 20 | 381 | 79 | 777 | 1632 | 2138 |
| Pre-chlorination 1,3,5-Trinitrobenzene | - | 4.3 | 93 | 6 | 18 | 23 | 99 |
| | - | 10.8 | 116 | 9 | 10 | 33 | 107 |
| | - | 21.6 | 86 | 15 | 17 | 35 | 145 |
| | - | 43.0 | 92 | 12 | 22 | 67 | 239 |
| | - | 107.0 | 74 | 15 | 23 | 85 | 307 |
| | + | 4.3 | 109 | 17 | 21 | 32 | 103 |
| | + | 10.8 | 127 | 10 | 17 | 26 | 112 |
| | + | 21.6 | 126 | 14 | 20 | 44 | 132 |
| | + | 43.0 | 115 | 7 | 35 | 34 | 173 |
| | + | 107.0 | 77 | 13 | 38 | 52 | 217 |

Table A-1 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Post-chlorination 1,3,5-Trinitrobenzene | - | 4.3 | 55 | 13 | 14 | 29 | 93 |
| | - | 10.8 | 62 | 14 | 13 | 36 | 119 |
| | - | 21.6 | 73 | 14 | 20 | 74 | 132 |
| | - | 43.0 | 93 | 23 | 24 | 40 | 191 |
| | - | 107.0 | 130 | 17 | 33 | 107 | 285 |
| | + | 4.3 | 136 | 11 | 27 | 25 | 104 |
| | + | 10.8 | 137 | 13 | 22 | 34 | 112 |
| | + | 21.6 | 156 | 18 | 20 | 33 | 129 |
| | + | 43.0 | 125 | 14 | 35 | 54 | 160 |
| | + | 107.0 | 147 | 20 | 25 | 70 | 193 |

Table A-2

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

1,3,5-TRINITROBENZENE

EXPERIMENT 25

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| **Negative control** | - | | 38 | 14 | 15 | 24 | 86 |
| | + | | 24 | 16 | 33 | 31 | 126 |
| **Positive controls** | | | | | | | |
| β-Propiolactone | - | 50 | 1500 | | | | |
| 9-Aminoacridine | - | 100 | | 700 | | | |
| 2-Nitrofluorene | - | 50 | | | 300 | | |
| AF2 | - | 0.1 | | | | 0 | 0 |
| 2-Anthramine | + | 20 | 600 | 600 | 2000 | 1200 | 2200 |
| **Pre-chlorination** | - | 44 | 49 | 14 | 122 | 121 | 354 |
| 1,3,5-Trinitrobenzene | + | 44 | 13 | 7 | 19 | 72 | 167 |
| **Post-chlorination** | - | 0.9 | 32 | 9 | 18 | 26 | 95 |
| 1,3,5-Trinitrobenzene | - | 1.8 | 32 | 15 | 15 | 31 | 114 |
| | - | 4.5 | 31 | 16 | 23 | 37 | 121 |
| | - | 9.0 | 30 | 17 | 33 | 79 | 173 |
| | - | 18.0 | 27 | 16 | 47 | 161 | 206 |
| | - | 44.0 | 33 | 34 | 129 | 115 | 347 |
| | + | 0.9 | 11 | 15 | 22 | 31 | 103 |
| | + | 1.8 | 15 | 9 | 21 | 39 | 103 |
| | + | 4.5 | 14 | 16 | 17 | 44 | 126 |
| | + | 9.0 | 12 | 9 | 26 | 31 | 139 |
| | + | 18.0 | 21 | 12 | 23 | 45 | 148 |
| | + | 44.0 | 25 | 12 | 28 | 81 | 163 |

Table A-3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 26

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 50 | 20 | 41 | 28 | 144 |
| | + | | 19 | 35 | 43 | 54 | 137 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1500 | | | | |
| 9-Aminoacridine | - | 100 | | 440 | | | |
| 2-Nitrofluorene | - | 50 | | | 324 | | |
| AF2 | - | 0.1 | | | | 600 | 1500 |
| 2-Anthramine | + | 20 | 217 | 500 | 3000 | 1000 | 6000 |
| Pre-ozonation | - | 94 | 42 | 23 | 38 | 60 | 176 |
| 1,3,5-Trinitrobenzene | + | 94 | 31 | 28 | 33 | 34 | 179 |
| Post-ozonation | - | 1.8 | 17 | 25 | 19 | 24 | 112 |
| 1,3,5-Trinitrobenzene | - | 3.6 | 13 | 29 | 24 | 36 | 130 |
| | - | 9.0 | 17 | 30 | 14 | 37 | 137 |
| | - | 18.0 | 18 | 23 | 18 | 34 | 156 |
| | - | 36.0 | 21 | 11 | 23 | 48 | 128 |
| | - | 90.0 | 48 | 16 | 28 | 60 | 143 |
| | + | 1.8 | 30 | 27 | 44 | 44 | 120 |
| | + | 3.6 | 17 | 17 | 39 | 44 | 158 |
| | + | 9.0 | 19 | 15 | 48 | 52 | 126 |
| | + | 18.0 | 28 | 27 | 44 | 43 | 142 |
| | + | 36.0 | 18 | 17 | 30 | 45 | 150 |
| | + | 90.0 | 18 | 25 | 34 | 40 | 133 |

Table A-4

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 35

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 30 | 8 | 13 | 22 | 73 |
| | + | | 18 | 12 | 19 | 39 | 71 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1050 | | | | |
| 9-Aminoacridine | - | 100 | | 218 | | | |
| 2-Nitrofluorene | - | 50 | | | 2000 | | |
| AF2 | - | 0.1 | | | | 24 | 73 |
| 2-Anthramine | + | 20 | 500 | 1270 | 2300 | 2540 | 1875 |
| Pre-ozonation | - | 91 | 6 | 52 | 854 | C* | 0 |
| 1,3,5-Trinitrobenzene | + | 91 | 40 | 13 | 65 | C | 237 |
| Post-ozonation | - | 18 | 42 | 15 | 177 | 337 | 301 |
| 1,3,5-Trinitrobenzene | - | 35 | 36 | 75 | 494 | 877 | 520 |
| | - | 53 | 2 | C | 787 | 764 | 609 |
| | - | 71 | 3 | 147 | 503 | 1124 | 702 |
| | - | 88 | 28 | 8 | 370 | 163 | 250 |
| | + | 18 | 20 | 26 | 22 | 63 | 139 |
| | + | 35 | 21 | 9 | 36 | 100 | 140 |
| | + | 53 | 11 | 12 | 35 | 130 | 164 |
| | + | 71 | C | 12 | 54 | 100 | 231 |
| | + | 88 | 26 | 10 | C | 27 | 276 |

* C, contaminated.

Table A-5

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,5-TRINITROBENZENE

EXPERIMENT 44

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1.0 | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Pre-ozonation | - | 91 | 0 | 131 | 0 | 307 | 15K* |
| 1,3,5-Trinitrobenzene | + | 91 | 18 | 13 | 68 | 77 | 361 |
| Post-ozonation | - | 18 | 20 | 16 | 160 | 108 | 435 |
| 1,3,5-Trinitrobenzene | - | 35 | 15 | 67 | 682 | 664 | 783 |
| | - | 53 | 0 | 172 | 1338 | 895 | 1223 |
| | - | 71 | 0 | 223 | 1016 | 1160 | 1248 |
| | - | 88 | 0 | 203 | 365 | 213 | 0K |
| | + | 18 | 9 | 8 | 14 | 37 | 186 |
| | + | 35 | 15 | 5 | 16 | 35 | 279 |
| | + | 53 | 13 | 8 | 24 | 66 | 290 |
| | + | 71 | 19 | 17 | 52 | 62 | 372 |
| | + | 88 | 20 | 25 | 59 | 104 | 436 |

* K, killing.

Table A-6

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

1,3-DINTROBENZENE

EXPERIMENT 30

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 |
|---|---|---|---|---|---|---|
| Negative control | - | | * | 13 | 12 | 27 |
| | + | | 22 | 9 | 21 | 34 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 | * | | | |
| 9-Aminoacridine | - | 100 | | 910 | | |
| 2-Nitrofluorene | - | 50 | | | 230 | |
| AF2 | - | 0.1 | | | | 320 |
| 2-Anthramine | + | 20 | 100 | 325 | 330 | 142 |
| Pre-chlorination | - | 46.00 | 54 | * | 26 | * |
| 1,3-Dinitrobenzene | + | 46.00 | 18 | 20 | * | 41 |
| Post-chlorination | - | 0.92 | 44 | 15 | 16 | 26 |
| 1,3,-Dinitrobenzene | - | 1.84 | 53 | 16 | 28 | 29 |
| | - | 4.60 | 59 | 18 | 22 | 24 |
| | - | 9.20 | 40 | * | 27 | 42 |
| | - | 18.40 | 39 | * | 19 | 41 |
| | - | 46.00 | 53 | * | 21 | * |
| | + | 0.92 | 14 | * | 19 | * |
| | + | 1.84 | 14 | 21 | 20 | * |
| | + | 4.60 | 18 | 37 | 21 | * |
| | + | 9.20 | 12 | 22 | 16 | 42 |
| | + | 18.40 | 26 | 31 | * | 44 |
| | + | 46.00 | 24 | 23 | * | 32 |

* Problem with plates and media.

Table A-7

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3,-DINITROBENZENE

EXPERIMENT 39

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 67 | 11 | 22 | 32 | 92 |
| | + | | 102 | 13 | 16 | 29 | 114 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 157 | | | | |
| 9-Aminoacridine | - | 100 | | 1252 | | | |
| 2-Nitrofluorene | - | 50 | | | 9 | | |
| AF2 | - | 0.1 | | | | 218 | 554 |
| 2-Anthramine | + | 20 | 381 | 79 | 777 | 1632 | 2138 |
| Pre-chlorination | - | 41.0 | 70 | 12 | 40 | 61 | 89 |
| 1,3-Dinitrobenzene | + | 41.0 | 93 | 15 | 19 | 33 | 83 |
| Post-chlorination | - | 1.6 | 74 | 5 | 16 | 22 | 89 |
| 1,3,-Dinitrobenzene | - | 4.0 | 51 | 11 | 15 | 36 | 108 |
| | - | 8.0 | 72 | 8 | 19 | 31 | 75 |
| | - | 16.0 | 76 | 17 | 22 | 40 | 96 |
| | - | 41.0 | 68 | 15 | 25 | 62 | 94 |
| | + | 1.6 | 85 | 16 | 25 | 31 | 103 |
| | + | 4.0 | 101 | 16 | 24 | 38 | 85 |
| | + | 8.0 | 86 | 12 | 23 | 38 | 100 |
| | + | 16.0 | 125 | 9 | 15 | 37 | 115 |
| | + | 41.0 | 104 | 15 | 23 | 44 | 99 |

Table A-8

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE

EXPERIMENT 42

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 26 | 6 | 14 | 17 | 127 |
| | + | | 13 | 12 | 20 | 30 | 96 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 42 | | | | |
| 9-Aminoacridine | - | 100 | | 536 | | | |
| 2-Nitrofluorene | - | 50 | | | 1633 | | |
| AF2 | - | 0.1 | | | | 293 | 997 |
| 2-Anthramine | - | 2.5 | 18 | 5 | 14 | | 134 |
| | + | 2.5 | 87 | 27 | 437 | 387 | 634 |
| Pre-chlorination | - | 41 | 21 | 2 | 28 | 27 | 125 |
| 1,3-Dinitrobenzene | + | 41 | 12 | 7 | 17 | 21 | 120 |
| Post-chlorination | - | 8 | 26 | 4 | 17 | 13 | 110 |
| 1,3-Dinitrobenzene | - | 16 | 17 | 4 | 16 | 17 | 138 |
| | - | 24 | 24 | 5 | 15 | 20 | 116 |
| | - | 32 | 22 | 4 | 19 | 30 | 138 |
| | - | 41 | 14 | 3 | 17 | 29 | 126 |
| | + | 8 | 8 | 5 | 18 | 30 | 112 |
| | + | 16 | 12 | 5 | 21 | 30 | 138 |
| | + | 24 | 11 | 5 | 15 | 17 | 110 |
| | + | 32 | 11 | 9 | 16 | 28 | 132 |
| | + | 41 | 9 | 5 | 17 | 17 | 137 |

Table A-9

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

1,3-DINITROBENZENE

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 34 | 7 | 12 | 14 | 82 |
| | + | | 14 | 6 | 24 | 25 | 96 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 547 | | | | |
| 9-Aminoacridine | - | 100 | | 400 | | | |
| 2-Nitrofluorene | - | 50 | | | 700 | | |
| AF2 | - | 0.1 | | | | 200 | 300 |
| 2-Anthramine | + | 20 | 108 | 11 | 54 | 300 | 1200 |
| Pre-ozonation 1,3-Dinitrobenzene | - | 13.0 | 23 | 2 | 24 | 42 | 107 |
| | + | 13.0 | 11 | 10 | 36 | 25 | 99 |
| Post-ozonation 1,3-Dinitrobenzene | - | 0.2 | 24 | | 17 | 26 | 99 |
| | - | 0.4 | 27 | 11 | 21 | 24 | 92 |
| | - | 1.0 | 28 | 5 | 12 | 30 | 76 |
| | - | 1.9 | 29 | 7 | 12 | 23 | 87 |
| | - | 3.9 | 34 | 6 | 13 | 37 | 77 |
| | - | 9.7 | 35 | 8 | 29 | 33 | 86 |
| | + | 0.2 | 10 | 11 | 36 | 43 | 103 |
| | + | 0.4 | 11 | 9 | 39 | 31 | 86 |
| | + | 1.0 | 17 | 7 | 39 | 33 | 82 |
| | + | 1.9 | 19 | 8 | 33 | 23 | 88 |
| | + | 3.9 | 21 | 7 | 39 | 21 | 72 |
| | + | 9.7 | 21 | 9 | 45 | 25 | 85 |

Table A-10

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE

EXPERIMENT 36

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 |
|---|---|---|---|---|---|---|
| **Negative control** | - | | 28 | 5 | 9 | 22 |
| | + | | 14 | 2 | 15 | 21 |
| **Positive controls** | | | | | | |
| β-Propiolactone | - | 50 | 224 | | | |
| 9-Aminoacridine | - | 100 | | 900 | | |
| 2-Nitrofluorene | - | 50 | | | 1300 | |
| AF2 | - | 0.1 | | | | 451 |
| 2-Anthramine | + | 20 | 416 | 322 | 1955 | 2200 |
| **Pre-ozonation** | - | 27.0 | 15 | 6 | 18 | 23 |
| 1,3-Dinitrobenzene | + | 27.0 | 7 | 3 | 8 | 20 |
| **Post-ozonation** | - | 4.6 | 25 | 4 | 11 | 18 |
| 1,3-Dinitrobenzene | - | 9.2 | 33 | 5 | 20 | 23 |
| | - | 13.8 | 21 | 8 | 12 | 25 |
| | - | 18.4 | 27 | 9 | 25 | 24 |
| | - | 23.0 | 17 | 5 | 21 | 43 |
| | + | 4.6 | 15 | 3 | 7 | 22 |
| | + | 9.2 | 20 | 3 | 6 | 15 |
| | + | 13.8 | 7 | 5 | 10 | 9 |
| | + | 18.4 | 5 | 5 | 7 | 19 |
| | + | 23.0 | 6 | 2 | 4 | 7 |

Table A-11

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
1,3-DINITROBENZENE

EXPERIMENT 40

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative control | - | | 115 |
| | + | | 119 |
| Positive controls | | | |
| AF2 | - | 0.1 | 446 |
| 2-Anthramine | + | 10 | 1755 |
| Pre-ozonation | - | 12.8 | 137 |
| 1,3-Dinitrobenzene | + | 12.8 | 175 |
| Post-ozonation | - | 2.6 | 156 |
| 1,3-Dinitrobenzene | - | 5.1 | 144 |
| | - | 7.7 | 140 |
| | - | 10.2 | 149 |
| | - | 12.8 | 145 |
| | + | 2.6 | 122 |
| | + | 5.1 | 139 |
| | + | 7.7 | 123 |
| | + | 10.2 | 143 |
| | + | 12.8 | 134 |

Table A-12

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
TRINITROGLYCERINE*

EXPERIMENT 25

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 38 | 14 | 15 | 24 | 86 |
| | + | | 24 | 16 | 33 | 31 | 126 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1500 | | | | |
| 9-Aminoacridine | - | 100 | | 700 | | | |
| 2-Nitrofluorene | - | 50 | | | 300 | | |
| AF2 | - | 0.1 | | | | 0 | 0 |
| 2-Anthramine | + | 20 | 600 | 600 | 2000 | 1200 | 2200 |
| Pre-chlorination | - | 0.25 ml | 35 | 15 | 16 | 26 | 119 |
| Trinitroglycerine | + | 0.25 | 18 | 17 | 13 | 25 | 80 |
| Post-chlorination | - | 0.05 ml | 26 | 18 | 16 | 23 | 110 |
| Trinitroglycerine | - | 0.01 | 31 | 9 | 23 | 19 | 83 |
| | - | 0.025 | 40 | 10 | 16 | 21 | 90 |
| | - | 0.05 | 36 | 15 | 16 | 20 | 101 |
| | - | 0.1 | 38 | 10 | 13 | 34 | 105 |
| | - | 0.25 | 26 | 14 | 23 | 24 | 126 |
| | + | 0.05 | 18 | 9 | 15 | 30 | 115 |
| | + | 0.01 | 15 | 14 | 17 | 25 | 93 |
| | + | 0.025 | 19 | 11 | 14 | 32 | 89 |
| | + | 0.05 | 17 | 7 | 15 | 31 | 93 |
| | + | 0.1 | 13 | 9 | 17 | 34 | 73 |
| | + | 0.25 | 23 | 10 | 21 | 18 | 84 |

* Saturated solution.

Table A-13

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

TRINITROGLYCERINE*

EXPERIMENT 34

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 |
|---|---|---|---|---|---|---|
| Negative control | - | | 52 | 16 | 13 | 38 |
| | + | | 87 | 17 | 9 | 57 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 µg | 1590 | | | |
| 9-Aminoacridine | - | 100 | | 475 | | |
| 2-Nitrofluorene | - | 50 | | | 2054 | |
| AF2 | - | 0.1 | | | | 46 |
| 2-Anthramine | + | 20 | 516 | 760 | 2800 | 3000 |
| Pre-chlorination | - | 0.25 ml | 52 | 17 | 17 | 30 |
| Trinitroglycerine | + | 0.25 | 78 | 9 | 17 | 25 |
| Post-chlorination | - | 0.05 ml | 50 | 12 | 18 | 33 |
| Trinitroglycerine | - | 0.1 | 69 | 8 | 14 | 30 |
| | - | 0.15 | 42 | 16 | 15 | 31 |
| | - | 0.2 | 41 | 8 | 18 | 26 |
| | - | 0.25 | 60 | 18 | 12 | 21 |
| | + | 0.05 | 53 | 24 | 23 | 42 |
| | + | 0.1 | 55 | 20 | C† | 40 |
| | + | 0.15 | 55 | 18 | 22 | 41 |
| | + | 0.2 | 72 | 18 | 16 | 25 |
| | + | 0.25 | 70 | 14 | 17 | 33 |

* Saturated solution.

† C, contaminated.

Table A-14

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
TRINITROGLYCERINE*

EXPERIMENT 40

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative control | - | | 115 |
| | + | | 119 |
| Positive controls | | | |
| AF2 | - | 0.1 µg | 446 |
| 2-Anthramine | + | 10 | 1755 |
| Pre-chlorination | - | 0.25 ml | 105 |
| Trinitroglycerine | + | 0.25 | 130 |
| Post-chlorination | - | 0.05 ml | 127 |
| Trinitroglycerine | - | 0.1 | 146 |
| | - | 0.15 | 138 |
| | - | 0.2 | 123 |
| | - | 0.25 | 120 |
| | + | 0.05 | 118 |
| | + | 0.1 | 123 |
| | + | 0.15 | 93 |
| | + | 0.2 | 139 |
| | + | 0.25 | 115 |

* Saturated solution.

Table A-15

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 35 | 16 | 29 | 38 | 100 |
| | + | | 24 | 18 | 33 | 30 | 133 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1740 | | | | |
| 9-Aminoacridine | - | 100 | | 168 | | | |
| 2-Nitrofluorene | - | 50 | | | 200 | | |
| AF2 | - | 0.1 | | | | 236 | 1200 |
| 2-Anthramine | + | 20 | T† | 432 | ~6000 | 840 | ~8000 |
| Pre-chlorination | - | 0.25 ml | 36 | 16 | 15 | 21 | 108 |
| PETN | + | 0.25 | 20 | 17 | 35 | 38 | 106 |
| Post-chlorination | - | 0.005 ml | 27 | 11 | 24 | 24 | 109 |
| PETN | - | 0.01 | 32 | 13 | 17 | 30 | 103 |
| | - | 0.025 | 36 | C‡ | 24 | 21 | 105 |
| | - | 0.05 | 40 | 15 | 17 | 20 | 100 |
| | - | 0.1 | 31 | 15 | 9 | 16 | 108 |
| | - | 0.25 | 33 | 17 | 13 | 31 | 95 |
| | + | 0.005 | 16 | 10 | 29 | 40 | 84 |
| | + | 0.01 | 14 | 16 | 37 | 38 | 106 |
| | + | 0.025 | 20 | 9 | 26 | 37 | 87 |
| | + | 0.05 | 21 | 10 | 31 | 56 | 87 |
| | + | 0.1 | 15 | 12 | 16 | 37 | 106 |
| | + | 0.25 | 23 | 9 | 27 | 39 | 120 |

* Saturated solution.
† T, toxic.
‡ C, contaminated.

Table A-16

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 39

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 67 | 11 | 22 | 32 | 92 |
| | + | | 102 | 13 | 16 | 28 | 114 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 157 | | | | |
| 9-Aminoacridine | - | 100 | | 1252 | | | |
| 2-Nitrofluorene | - | 50 | | | 9 | | |
| AF2 | - | 0.1 | | | | 218 | 554 |
| 2-Anthramine | + | 20 | 381 | 79 | 777 | 1632 | 2138 |
| Pre-chlorination | - | 0.25 ml | 85 | 16 | 11 | 31 | 77 |
| PETN | + | 0.25 ml | 128 | 15 | 14 | 44 | 95 |
| Post-chlorination | - | 0.01 | 75 | 11 | 14 | 23 | 94 |
| PETN | - | 0.025 | 111 | 13 | 10 | 21 | 79 |
| | - | 0.05 | 95 | 19 | 9 | 24 | 82 |
| | - | 0.1 | 86 | 18 | 13 | 25 | 90 |
| | - | 0.25 | 93 | 18 | 11 | 24 | 83 |
| | + | 0.01 | 118 | 14 | 15 | 27 | 104 |
| | + | 0.025 | 126 | 13 | 9 | 33 | 93 |
| | + | 0.05 | 127 | 15 | 15 | 42 | 111 |
| | + | 0.1 | 141 | 18 | 15 | 39 | 107 |
| | + | 0.25 | 156 | 10 | 13 | 32 | 128 |

* Saturated solution.

Table A-17

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 26

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 50 | 20 | 41 | 28 | 144 |
| | + | | 19 | 35 | 43 | 54 | 137 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1500 | | | | |
| 9-Aminoacridine | - | 100 | | 440 | | | |
| 2-Nitrofluorene | - | 50 | | | 324 | | |
| AF2 | - | 0.1 | | | | 600 | 1500 |
| 2-Anthramine | + | 20 | 217 | 500 | 3000 | 1000 | 6000 |
| Pre-ozonation | - | 0.25 ml | 39 | 13 | 24 | 30 | 144 |
| PETN | + | 0.25 ml | 27 | 15 | 24 | 54 | 132 |
| Post-ozonation | - | 0.005 ml | 47 | 14 | 17 | 39 | 126 |
| PETN | - | 0.01 | 66 | 15 | 22 | 40 | 132 |
| | - | 0.025 | 54 | 21 | 22 | 27 | 144 |
| | - | 0.05 | 44 | 18 | 19 | 40 | 134 |
| | - | 0.1 | 52 | 8 | 27 | 32 | 137 |
| | - | 0.25 | 68 | 15 | 21 | 40 | 121 |
| | + | 0.005 | 16 | 25 | 37 | 41 | 131 |
| | + | 0.01 | 22 | 19 | 33 | 46 | 146 |
| | + | 0.025 | 15 | 18 | 40 | 60 | 142 |
| | + | 0.05 | 21 | 31 | 36 | 51 | 134 |
| | + | 0.1 | 19 | 13 | 32 | 52 | 156 |
| | + | 0.25 | 26 | 23 | 28 | 34 | 149 |

* Saturated solution.

Table A-18

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 35

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 30 | 8 | 13 | 22 | 73 |
| | + | | 18 | 12 | 19 | 39 | 71 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1050 | | | | |
| 9-Aminoacridine | - | 100 | | 218 | | | |
| 2-Nitrofluorene | - | 50 | | | 2000 | | |
| AF2 | - | 0.1 | | | | 24 | 73 |
| 2-Anthramine | + | 20 | 500 | 1270 | 2300 | 2540 | 1875 |
| Pre-ozonation | - | 0.25 ml | 15 | 2 | 11 | 14 | 78 |
| PETN | + | 0.25 | 11 | 6 | 17 | 23 | 85 |
| Post-ozonation | - | 0.05 ml | 15 | 10 | 14 | 26 | 62 |
| PETN | - | 0.1 | 22 | 2 | 6 | 35 | 85 |
| | - | 0.15 | C† | 5 | 13 | 29 | 72 |
| | - | 0.2 | 20 | 9 | 3 | 18 | 68 |
| | - | 0.25 | C | 4 | 15 | 26 | 74 |
| | + | 0.05 | 15 | 10 | 25 | 20 | 58 |
| | + | 0.1 | 18 | 12 | 15 | 17 | 89 |
| | + | 0.15 | 16 | 5 | 13 | 29 | 78 |
| | + | 0.2 | 11 | 9 | 24 | 29 | 70 |
| | + | 0.25 | 8 | 8 | 24 | 33 | 71 |

* Saturated solution.
† C, contaminated.

Table A-19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
CONDENSATE WATER

EXPERIMENT 29

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1538 | TA98 |
|---|---|---|---|---|---|
| Negative control | - | | 35 | 12 | 16 |
| | + | | 5 | 14 | 40 |
| Positive controls | | | | | |
| β -Propiolactone | - | 50 | 1000 | | |
| 2-Nitrofluorene | - | 50 | | 1200 | |
| AF2 | - | 0.1 | | | 28 |
| 2-Anthramine | + | 20 | 325 | 480 | 1400 |
| Pre-chlorination | - | 17 | 17 | 38 | 37 |
| Condensate water | + | 17 | 26 | 27 | 43 |
| Post-chlorination | - | 3.2 | 31 | 22 | 27 |
| Condensate water | - | 6.4 | 13 | 23 | 35 |
| | - | 9.6 | 30 | 55 | 35 |
| | - | 12.8 | 26 | 49 | 73 |
| | - | 16 | 25 | 77 | 67 |
| | + | 3.2 | 25 | 20 | 30 |
| | + | 6.4 | 10 | 20 | 25 |
| | + | 9.6 | 10 | 26 | 51 |
| | + | 12.8 | 7 | 32 | 44 |
| | + | 16 | 14 | 27 | 55 |

Table A-20

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
CONDENSATE WATER

EXPERIMENT 44

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA98 | TA100 |
|---|---|---|---|---|
| Negative control | - | | 22 | 133 |
| | + | | 26 | 139 |
| Positive controls | | | | |
| AF2 | - | 0.1 | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 705 | 998 |
| Pre-chlorination | - | 16.8 | 24 | 148 |
| Condensate water | + | 16.8 | 26 | 163 |
| Post-chlorination | - | 3.4 | 14 | 132 |
| Condensate water | - | 6.7 | 18 | 144 |
| | - | 10.1 | 14 | 137 |
| | - | 13.5 | 25 | 160 |
| | - | 16.8 | 24 | 134 |
| | + | 3.4 | 17 | 123 |
| | + | 6.7 | 25 | 135 |
| | + | 10.1 | 24 | 149 |
| | + | 13.5 | 27 | 134 |
| | + | 16.8 | 25 | 145 |

Table A-21

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
SYRINGALDAZINE

EXPERIMENT 20

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 35 | 15 | 16 | 52 | 120 |
| | + | | 30 | 15 | 50 | 52 | 104 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 800 | | | | |
| 9-Aminoacridine | - | 100 | | 1500 | | | |
| 2-Nitrofluorene | - | 50 | | | 500 | | |
| AF2 | - | 0.1 | | | | 450 | 1500 |
| 2-Anthramine | + | 20 | 850 | 500 | 3000 | 3000 | 3000 |
| Pre-chlorination | - | 30 | 37 | 10 | 12 | 54 | 128 |
| Syringaldazine | + | 30 | 43 | 19 | 20 | 43 | 104 |
| Post-chlorination | - | 0.003 | 31 | 9 | 25 | 62 | 118 |
| Syringaldazine | - | 0.006 | 32 | 13 | 20 | 42 | 94 |
| | - | 0.016 | 43 | 11 | 24 | 34 | 129 |
| | - | 0.03 | 32 | 7 | 20 | 54 | 112 |
| | - | 0.06 | 34 | 9 | 25 | 16 | 152 |
| | - | 0.16 | 43 | 17 | 18 | 21 | 124 |
| | + | 0.003 | 100 | 6 | 30 | 45 | 119 |
| | + | 0.006 | 35 | 13 | 40 | 45 | 104 |
| | + | 0.016 | 30 | 11 | 35 | 54 | 120 |
| | + | 0.03 | 28 | 24 | 23 | 44 | 78 |
| | + | 0.06 | 43 | K* | 22 | 33 | 84 |
| | + | 0.16 | 50 | 14 | 26 | 18 | 102 |

* K, killing.

Table A-22

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
SYRINGALDAZINE

EXPERIMENT 33

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 6 | 5 | 21 | 98 |
| | + | | 17 | 7 | 16 | 26 | 106 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 950 | | | | |
| 9-Aminoacridine | - | 100 | | 239 | | | |
| 2-Nitrofluorene | - | 50 | | | 1260 | | |
| AF2 | - | 0.1 | | | | 21 | 137 |
| 2-Anthramine | + | 20 | 324 | 14 | 585 | 2400 | 1530 |
| Pre-chlorination | - | 31 | 46 | 9 | 13 | 14 | 87 |
| Syringaldazine | + | 31 | 44 | 5 | 16 | 21 | 91 |
| Post-chlorination | - | 4 | 44 | 2 | 3 | 19 | 100 |
| Syringaldazine | - | 8 | 51 | 4 | 9 | 21 | 95 |
| | - | 12 | 46 | 3 | 6 | 14 | 96 |
| | - | 16 | 33 | 5 | 10 | 18 | 94 |
| | - | 20 | 46 | 4 | 8 | 15 | 77 |
| | + | 4 | 31 | 6 | 14 | 20 | 97 |
| | + | 8 | 37 | 10 | 14 | 23 | 80 |
| | + | 12 | 32 | 5 | 15 | 24 | 72 |
| | + | 16 | 28 | 4 | 16 | 20 | 81 |
| | + | 20 | 42 | 4 | 11 | 16 | 71 |

Table A-23

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
HMX*

EXPERIMENT 21

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA100 |
|---|---|---|---|---|---|
| Negative control | - | | 77 | 14 | 147 |
| | + | | 53 | 29 | 166 |
| Positive controls | | | | | |
| β-Propiolactone | - | 50 µg | 2400 | | |
| 9-Aminoacridine | - | 100 | | 2115 | |
| AF2 | - | 0.1 | | | 188 |
| 2-Anthramine | + | 20 | 253 | 1305 | ~6000 |
| Pre-chlorination | - | 0.25 ml | 72 | 14 | 151 |
| HMX | + | 0.25 | 35 | 29 | 153 |
| Post-chlorination | - | 0.005 ml | 88 | 14 | 165 |
| HMX | - | 0.01 | 87 | 20 | 158 |
| | - | 0.025 | 86 | 34 | 195 |
| | - | 0.05 | 91 | 27 | 144 |
| | - | 0.1 | 93 | † | † |
| | - | 0.25 | † | † | † |
| | + | 0.005 | 35 | 26 | 151 |
| | + | 0.01 | 45 | 28 | 147 |
| | + | 0.025 | 76 | 34 | 135 |
| | + | 0.05 | 78 | † | 145 |
| | + | 0.1 | 43 | † | 161 |
| | + | 0.25 | † | † | † |

* Saturated solution.
† Problem with plates or media.

Table A-24

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
HMX*
EXPERIMENT 30

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 12 | 27 | 131 |
| | + | | 9 | 21 | 34 | 84 |
| Positive controls | | | | | | |
| 9-Aminoacridine | - | 100 µg | 910 | | | |
| 2-Nitrofluorene | - | 50 | | 230 | | |
| AF2 | - | 0.1 | | | 320 | 800 |
| 2-Anthramine | + | 20 | 325 | 330 | 142 | 710 |
| Pre-chlorination | - | 0.25 ml | † | 11 | 39 | 94 |
| HMX | + | 0.25 | 21 | 6 | 38 | 86 |
| Post-chlorination | - | 0.005 ml | 10 | 11 | 27 | 150 |
| HMX | - | 0.01 | 18 | 14 | 28 | 142 |
| | - | 0.025 | 13 | 12 | 27 | 138 |
| | - | 0.05 | † | 16 | 30 | 165 |
| | - | 0.1 | † | 16 | 42 | 144 |
| | - | 0.25 | † | 11 | 39 | 102 |
| | + | 0.005 | 22 | 11 | 33 | † |
| | + | 0.01 | 20 | 12 | 44 | † |
| | + | 0.025 | 21 | 11 | 48 | † |
| | + | 0.05 | 21 | 17 | 47 | 86 |
| | + | 0.1 | 24 | C‡ | 51 | 121 |
| | + | 0.25 | 34 | 8 | 48 | 91 |

* Saturated solution.
† Problem with media or plates.
‡ C, contaminated.

Table A-25

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
HMX*

EXPERIMENT 37

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 7 | 10 | 11 | 14 | 91 |
| | + | | 5 | 7 | 9 | 19 | 77 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 262 | | | | |
| 9-Aminoacridine | - | 100 | | 1405 | | | |
| 2-Nitrofluorene | - | 50 | | | 1130 | | |
| AF2 | - | 0.1 | | | | 400 | 1176 |
| 2-Anthramine | + | 20 | 330 | 247 | 1825 | 1836 | 1290 |
| Pre-chlorination | - | 0.25 ml | 9 | 3 | 7 | 11 | 70 |
| HMX | + | 0.25 | 5 | 8 | 5 | 13 | 74 |
| Post-chlorination | - | 0.05 ml | 6 | 2 | 5 | 17 | 63 |
| HMX | - | 0.1 | 11 | 2 | 5 | 10 | 84 |
| | - | 0.15 | 6 | 6 | 5 | 11 | 65 |
| | - | 0.2 | 10 | 6 | 9 | 21 | 74 |
| | - | 0.25 | 9 | 3 | 6 | 10 | 68 |
| | + | 0.05 | 7 | 6 | 5 | 20 | 85 |
| | + | 0.1 | 5 | 2 | 5 | 25 | 82 |
| | + | 0.15 | 2 | 3 | 12 | 9 | 94 |
| | + | 0.2 | 5 | 2 | 7 | 16 | 97 |
| | + | 0.25 | 8 | 3 | 7 | 11 | 79 |

* Saturated solution.

Table A-26

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
RDX

EXPERIMENT 21

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 77 | 14 | 42 | 64 | 147 |
| | + | | 53 | 29 | 41 | 81 | 166 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 2460 | | | | |
| 9-Aminoacridine | - | 100 | | 2115 | | | |
| 2-Nitrofluorene | - | 50 | | | 361 | | |
| AF2 | - | 0.1 | | | | 77 | 188 |
| 2-Anthramine | + | 20 | 253 | 1305 | 6870 | ~5000 | ~6000 |
| Pre-chlorination RDX | - | 12 | 71 | 15 | 33 | 56 | 149 |
| | + | 12 | 37 | 7 | 37 | C* | 136 |
| Post-chlorination RDX | - | 0.24 | 66 | 9 | 44 | 70 | 127 |
| | - | 0.48 | 40 | 17 | 38 | 48 | 128 |
| | - | 1.20 | 51 | 14 | 42 | 64 | 148 |
| | - | 2.40 | 67 | 16 | 41 | 58 | 162 |
| | - | 4.80 | 68 | 15 | 37 | 57 | 133 |
| | - | 12.00 | 63 | 16 | 39 | 56 | 167 |
| | + | 0.24 | 23 | 15 | 42 | 54 | 126 |
| | + | 0.48 | 49 | 18 | 47 | 57 | 139 |
| | + | 1.20 | 72 | 16 | 45 | 55 | 136 |
| | + | 2.40 | 44 | 22 | 49 | 62 | 178 |
| | + | 4.80 | 46 | 16 | 43 | 60 | 174 |
| | + | 12.00 | 43 | 24 | 47 | 43 | 133 |

* C, contaminated.

Table A-27

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
RDX

EXPERIMENT 29

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1538 | TA98 |
|---|---|---|---|---|---|
| Negative control | - | | 35 | 12 | 16 |
| | + | | 5 | 14 | 40 |
| Positive controls | | | | | |
| β-Propiolactone | - | 50 | 1000 | | |
| 2-Nitrofluorene | - | 50 | | 1200 | |
| AF2 | - | 0.1 | | | 28 |
| 2-Anthramine | + | 20 | 325 | 480 | 1400 |
| Pre-chlorination | - | 14.0 | 12 | 11 | 28 |
| RDX | + | 14.0 | 20 | 10 | 16 |
| Post-chlorination | - | 2.9 | 20 | 15 | 33 |
| RDX | - | 5.8 | 23 | 10 | 12 |
| | - | 8.7 | 28 | 13 | 30 |
| | - | 11.6 | 15 | 18 | 16 |
| | - | 14.0 | 16 | 10 | 23 |
| | + | 2.9 | 14 | 12 | 15 |
| | + | 5.8 | 13 | 15 | 16 |
| | + | 8.7 | 22 | 14 | 20 |
| | + | 11.6 | 8 | 8 | 36 |
| | + | 14.0 | 8 | 17 | 19 |

Table A-28

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
RDX

EXPERIMENT 40

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 19 | 115 |
| | + | | 9 | 14 | 28 | 119 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 | 456 | | | |
| 9-Aminoacridine | - | 100 | | 546 | | |
| AF2 | - | 0.1 | | | 98 | 446 |
| 2-Anthramine | + | 10 | 604 | 444 | 2320 | 1755 |
| Pre-chlorination | - | 4.1 | 13 | 9 | 23 | 106 |
| RDX | + | 4.1 | 20 | 17 | 23 | 120 |
| Post-chlorination | - | 0.8 | 28 | 16 | 13 | 99 |
| RDX | - | 1.6 | 19 | 15 | 14 | 106 |
| | - | 2.5 | 19 | 22 | 27 | 107 |
| | - | 3.3 | 23 | 16 | 18 | 133 |
| | - | 4.1 | 17 | 16 | 26 | 104 |
| | + | 0.8 | 13 | 17 | 22 | 126 |
| | + | 1.6 | 8 | 14 | 25 | 127 |
| | + | 2.5 | 12 | 12 | 30 | 105 |
| | + | 3.3 | 5 | 7 | 25 | 121 |
| | + | 4.1 | 14 | 8 | 28 | 120 |

Table A-29

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 35 | 16 | 29 | 38 | 100 |
| | + | | 24 | 18 | 33 | 30 | 133 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1740 | | | | |
| 9-Aminoacridine | - | 100 | | 168 | | | |
| 2-Nitrofluorene | - | 50 | | | 200 | | |
| AF2 | - | 0.1 | | | | 236 | 1200 |
| 2-Anthramine | + | 20 | T† | 432 | 6000 | 840 | 8000 |
| Pre-chlorination | - | 0.25 ml | 35 | 13 | 18 | 32 | 162 |
| DPO | + | 0.25 | 20 | 14 | 55 | 66 | 143 |
| Post-chlorination | - | 0.005 ml | 39 | 17 | 20 | C‡ | 145 |
| DPO | - | 0.01 | 31 | 17 | 12 | 27 | 119 |
| | - | 0.025 | 37 | 18 | 27 | 34 | 102 |
| | - | 0.05 | 34 | 15 | 27 | 26 | 141 |
| | - | 0.1 | 32 | 20 | 15 | 29 | 128 |
| | - | 0.25 | 41 | 16 | 24 | 29 | 126 |
| | + | 0.005 | 21 | 16 | 61 | 23 | 126 |
| | + | 0.01 | 19 | 16 | 64 | C | 159 |
| | + | 0.025 | 15 | 15 | 80 | 107 | 116 |
| | + | 0.05 | 18 | 14 | 75 | 85 | 133 |
| | + | 0.1 | 17 | 17 | 59 | 92 | 127 |
| | + | 0.25 | 28 | 16 | 71 | 129 | 165 |

* Photolyzed.
† T, toxic.
‡ C, contaminated.

Table A-30

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE

EXPERIMENT 33

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 37 | 6 | 5 | 21 | 98 |
| | + | | 17 | 7 | 16 | 26 | 106 |
| Positive controls | | | | | | | |
| 3-Propiolactone | - | 50 | 950 | | | | |
| 9-Aminoacridine | - | 100 | | 239 | | | |
| 2-Nitrofluorene | - | 50 | | | 1260 | | |
| AF2 | - | 0.1 | | | | 21 | 137 |
| 2-Anthramine | + | 20 | 324 | 14 | 585 | 2400 | 1530 |
| Pre-chlorination | - | 2.3 | 37 | 2 | 9 | 14 | 105 |
| DPO | - | 5.7 | 37 | 6 | 8 | 19 | 94 |
| | - | 11.5 | 36 | 9 | 9 | 21 | 96 |
| | - | 23 | 34 | 7 | 12 | 10 | 112 |
| | - | 57 | 44 | 6 | 12 | 61 | 97 |
| | + | 2.3 | 20 | 4 | 29 | 31 | 83 |
| | + | 5.7 | 20 | 5 | 56 | 64 | 88 |
| | + | 11.5 | 28 | 7 | 135 | 122 | 95 |
| | + | 23 | 14 | 7 | 232 | 223 | 96 |
| | + | 57 | 11 | 12 | 489 | 441 | 141 |

Table A-30 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 6 | 5 | 21 | 98 |
| | + | | 17 | 7 | 16 | 26 | 106 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 950 | | | | |
| 9-Aminoacridine | - | 100 | | 239 | | | |
| 2-Nitrofluorene | - | 50 | | | 1260 | | |
| AF2 | - | 0.1 | | | | 21 | 137 |
| 2-Anthramine | + | 20 | 324 | 14 | 585 | 2400 | 1530 |
| Post-chlorination DPO | - | 1.8 | 43 | 2 | 11 | 24 | 102 |
| | - | 4.5 | 34 | 7 | 5 | 20 | 98 |
| | - | 9.0 | 41 | 10 | 6 | 23 | 75 |
| | - | 18.0 | 48 | 5 | 5 | 27 | 115 |
| | - | 45.1 | 25 | 5 | 6 | 57 | 120 |
| | + | 1.8 | 14 | 2 | 38 | 35 | 92 |
| | + | 4.5 | 10 | 9 | 75 | 64 | 76 |
| | + | 9.0 | 7 | 11 | 114 | 134 | 98 |
| | + | 18.0 | 18 | 9 | 290 | 294 | 102 |
| | + | 45.1 | 15 | 16 | 395 | 312 | 78 |

Table A-31

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 19

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 25 | 9 | 29 | 53 | 131 |
| | + | | 26 | 9 | 30 | 68 | 126 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 900 | | | | |
| 9-Aminoacridine | - | 100 | | 500 | | | |
| 2-Nitrofluorene | - | 50 | | | 310 | | |
| AF2 | - | 0.1 | | | | 450 | 1000 |
| 2-Anthramine | + | 20 | 700 | 28 | 8920 | 3000 | 4000 |
| Pre-chlorination | - | 0.25 ml | 39 | 22 | 32 | 66 | 184 |
| DPS | + | 0.25 | 26 | 19 | 41 | 96 | 200 |
| Post-chlorination | - | 0.005 ml | 40 | 15 | 36 | 43 | 155 |
| DPS | - | 0.01 | 45 | 16 | 21 | 68 | 173 |
| | - | 0.025 | 51 | 16 | 29 | 61 | 232 |
| | - | 0.05 | 34 | 13 | 30 | 72 | 222 |
| | - | 0.1 | 39 | 14 | 29 | 71 | 282 |
| | - | 0.25 | 30 | 21 | 26 | 66 | 288 |
| | + | 0.005 | 17 | 20 | 29 | 73 | 218 |
| | + | 0.01 | 25 | 14 | 24 | 71 | 131 |
| | + | 0.025 | 20 | 11 | 19 | 72 | 203 |
| | + | 0.05 | 20 | 21 | 45 | 71 | 382 |
| | + | 0.1 | 32 | 28 | 53 | 94 | 200 |
| | + | 0.25 | 25 | 36 | 66 | 142 | 250 |

*Photolyzed.

Table A-32

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 35 | 16 | 29 | 38 | 100 |
| | + | | 24 | 18 | 33 | 30 | 133 |
| Postive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1740 | | | | |
| 9-Aminoacridine | - | 100 | | 168 | | | |
| 2-Nitrofluorene | - | 50 | | | 200 | | |
| AF2 | - | 0.1 | | | | 236 | 1200 |
| 2-Anthramine | + | 20 | T† | 432 | ~6000 | 840T | ~8000 |
| Pre-chlorination | - | 0.25 ml | 31 | 12 | 23 | 49 | 118 |
| DPS | + | 0.25 | 26 | 111 | 102 | 123 | 215 |
| Post-chlorination | - | 0.005 ml | 34 | 18 | 19 | 25 | 86 |
| DPS | - | 0.01 | 53 | 25 | 24 | 31 | 87 |
| | - | 0.025 | 41 | 17 | 23 | 20 | 105 |
| | - | 0.05 | 37 | 15 | 21 | 35 | 128 |
| | - | 0.1 | 34 | 12 | 26 | 42 | 144 |
| | - | 0.25 | 24 | 14 | 19 | 44 | 317 |
| | + | 0.005 | 17 | 24 | 29 | 41 | 140 |
| | + | 0.01 | 21 | 21 | 36 | 48 | 127 |
| | + | 0.025 | 14 | 35 | 59 | 70 | 141 |
| | + | 0.05 | 19 | 40 | 98 | 158 | 123 |
| | + | 0.1 | 23 | 71 | 116 | 146 | 172 |
| | + | 0.25 | 22 | 119 | 142 | 171 | 171 |

*Photolyzed.

†T, Toxic.

Table A-33

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

N,N-DIMETHYL-p-PHENYLEDIAMINE SULFATE

EXPERIMENT 33

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 37 | 6 | 5 | 21 | 98 |
| | + | | 17 | 7 | 16 | 26 | 106 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 950 | | | | |
| 9-Aminoacridine | - | 100 | | 239 | | | |
| 2-Nitrofluorene | - | 50 | | | 1260 | | |
| AF2 | - | 0.1 | | | | 21 | 137 |
| 2-Anthramine | + | 20 | 324 | 14 | 585 | 2400 | 1530 |
| Pre-chlorination | - | 1.4 | 29 | 1 | 11 | 18 | 109 |
| DPS | - | 3.5 | 35 | 3 | 15 | 19 | 92 |
| | - | 7 | 30 | 7 | 11 | 36 | 89 |
| | - | 14 | 26 | 11 | 12 | 45 | 82 |
| | - | 35 | 34 | 28 | 15 | 15 | 93 |
| | + | 1.4 | 10 | 10 | 87 | 87 | 110 |
| | + | 3.5 | 21 | 15 | 268 | 391 | 93 |
| | + | 7 | 14 | 20 | 457 | 531 | 117 |
| | + | 14 | 10 | 14 | 625 | 805 | 127 |
| | + | 35 | 8 | 42 | 901 | 958 | 147 |

Table A-33 (Concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 6 | 5 | 21 | 98 |
| | + | | 17 | 7 | 16 | 26 | 106 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 950 | | | | |
| 9-Aminoacridine | - | 100 | | 239 | | | |
| 2-Nitrofluorene | - | 50 | | | 1260 | | |
| AF2 | - | 0.1 | | | | 21 | 137 |
| 2-Anthramine | + | 20 | 324 | 14 | 585 | 2400 | 1530 |
| Post-chlorination | - | 1 | 41 | 2 | 12 | 23 | 109 |
| DPS | - | 2.5 | 29 | 2 | 7 | 21 | 96 |
| | - | 5 | 37 | 7 | 7 | 9 | 100 |
| | - | 10 | 27 | 6 | 11 | 29 | 85 |
| | - | 25 | 31 | 5 | 14 | 38 | 113 |
| | + | 1 | 13 | 9 | 18 | 27 | 102 |
| | + | 2.5 | 20 | 7 | 19 | 15 | 73 |
| | + | 5 | 13 | 6 | 13 | 17 | 83 |
| | + | 10 | 11 | 5 | 13 | 9 | 88 |
| | + | 25 | 13 | 6 | 11 | 12 | 93 |

Table A-34

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE

EXPERIMENT 45

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 15 | 16 | 32 | 147 |
| | + | | 18 | 28 | 42 | 141 |
| Positive controls | | | | | | |
| 9-Aminoacridine | - | 100 | 3141 | | | |
| 2-Nitrofluorene | - | 50 | | 1475 | | |
| AF2 | - | 0.1 | | | 139 | 885 |
| 2-Anthramine | - | 2.5 | 26 | 37 | 41 | 193 |
| | + | 2.5 | 277 | 2384 | 877 | 2334 |
| Pre-chlorination DPS | - | 6.7 | 14 | 25 | 27 | 164 |
| | - | 13.4 | 11 | 15 | 27 | 152 |
| | - | 20.1 | 23 | 23 | 24 | 158 |
| | - | 26.8 | 14 | 9 | 20 | 152 |
| | - | 33.5 | 14 | 13 | 23 | 157 |
| | + | 6.7 | 32 | 104 | 92 | 186 |
| | + | 13.4 | 21 | 182 | 168 | 167 |
| | + | 20.1 | 24 | 285 | 206 | 183 |
| | + | 26.8 | 26 | 308 | 282 | 185 |
| | + | 33.5 | 22 | 341 | 243 | 147 |

Table A-34 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Post-chlorination DPS | - | 5.7 | | 13 | 22 | 38 | 135 |
| | - | 11.4 | | 10 | 17 | 35 | 151 |
| | - | 17.1 | | 11 | 14 | 39 | 144 |
| | - | 22.8 | | 17 | 14 | 29 | 148 |
| | - | 28.5 | | 18 | 20 | 33 | 171 |
| | + | 5.7 | | 63 | 1410 | 1297 | 221 |
| | + | 11.4 | | 78 | 2562 | 1952 | 156 |
| | + | 17.1 | | 68 | 2574 | 2659 | 224 |
| | + | 22.8 | | 99 | 3186 | 3055 | 301 |
| | | 28.5 | | 148 | 3021 | 3266 | 311 |

Table A-35

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 29

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1537 | TA1538 | TA98 |
|---|---|---|---|---|---|
| Negative control | - | | 35 | 12 | 16 |
| | + | | 5 | 14 | 40 |
| Positive controls | | | | | |
| β-Propiolactone | - | 50 | 1000 | | |
| 2-Nitrofluorene | - | 50 | | 1200 | |
| AF2 | - | 0.1 | | | 28 |
| 2-Anthramine | + | 20 | 325 | 480 | 1400 |
| Pre-chlorination | - | 9 | 22 | 104 | C* |
| 7-50 LAP | + | 9 | 11 | 41 | 46 |
| Post-chlorination | - | 0.18 | 25 | 13 | 30 |
| 7-50 LAP | - | 0.36 | 19 | 14 | 41 |
| | - | 0.9 | 14 | 27 | 53 |
| | - | 1.8 | 28 | 58 | 78 |
| | - | 3.6 | 26 | 99 | 167 |
| | - | 9.0 | 30 | 233 | 308 |
| | + | 0.18 | 12 | 10 | 41 |
| | + | 0.36 | 11 | 62 | 31 |
| | + | 0.9 | 27 | 20 | 33 |
| | + | 1.8 | 11 | 31 | 37 |
| | + | 3.6 | 17 | 34 | 43 |
| | + | 9. | 26 | 76 | 88 |

* C, Contaminated.

Table A-36

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 38

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 95 | 11 | 13 | 23 | 121 |
| | + | | 130 | 12 | 19 | 29 | 128 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 298 | | | | |
| 9-Aminoacridine | - | 100 | | 1165 | | | |
| 2-Nitrofluorene | - | 50 | | | 882 | | |
| AF2 | - | 0.1 | | | | 300 | 840 |
| 2-Anthramine | + | 20 | 427 | 99 | 1866 | 2685 | 2910 |
| Pre-chlorination | - | 0.41 | 71 | 10 | 41 | 55 | 100 |
| 7-50 LAP | - | 0.82 | 112 | 16 | 80 | 65 | 104 |
| | - | 2.05 | 133 | 48 | 138 | 165 | 124 |
| | - | 4.1 | 127 | 38 | 236 | 249 | 169 |
| | - | 8.2 | 128 | 85 | 480 | 414 | 220 |
| | - | 21 | 161 | 158 | 775 | 920 | 374 |
| | + | 0.41 | 232 | 12 | 514 | 40 | 130 |
| | + | 0.82 | 213 | 14 | 40 | C* | 104 |
| | + | 2.05 | 192 | 24 | 323 | C* | 110 |
| | + | 4.1 | 236 | 16 | 248 | C* | 130 |
| | + | 8.2 | 199 | 28 | 55 | 59 | 147 |
| | + | 21 | 216 | 50 | 111 | 106 | 182 |

* C, Contaminated.

Table A-36 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Post-chlorination 7-50 LAP | - | 0.41 | 70 | 10 | 59 | 55 | 107 |
| | - | 0.82 | 86 | 11 | 104 | 80 | 110 |
| | - | 2.05 | 96 | 32 | 299 | 196 | 115 |
| | - | 4.1 | 96 | 54 | 429 | 285 | 124 |
| | - | 8.2 | 92 | 104 | 741 | 564 | 163 |
| | - | 21 | 65 | 160 | 968 | 1116 | 265 |
| | + | 0.41 | 130 | 8 | 23 | 30 | 53 |
| | + | 0.82 | 173 | 7 | 25 | 31 | 90 |
| | + | 2.05 | 150 | 5 | 33 | 31 | 69 |
| | + | 4.1 | 147 | 9 | 33 | 40 | 82 |
| | + | 8.2 | 144 | 8 | 47 | 65 | 87 |
| | + | 21 | 125 | K* | 105 | 153 | 192 |

* K, Killing.

Table A-37

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 44

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Pre-chlorination 7-50 LAP | - | 0.85 | C* | C | 157 | 94 | 189 |
| | - | 1.69 | 33 | 19 | 225 | 211 | 180 |
| | - | 2.54 | 29 | 35 | 308 | 224 | 224 |
| | - | 3.38 | 22 | 41 | 406 | 336 | 283 |
| | - | 4.23 | 19 | 64 | 340 | 405 | 287 |
| | + | 0.85 | 19 | 12 | 28 | 36 | 157 |
| | + | 1.69 | 17 | 15 | 40 | 41 | 178 C |
| | + | 2.54 | 11 | 12 | 38 C | 49 | 170 |
| | + | 3.38 | 15 | 13 | 46 | 55 | 175 C |
| | + | 4.23 | 15 C | 14 | 74 | 73 | 0 |

* C, Contaminated.

Table A-37 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Post-chlorination | - | 0.85 | 24 | 22 | 65 | 81 | 181 |
| 7-50 LAP | - | 1.69 | 30 | 27C | 126 | 107 | 161 |
| | - | 2.54 | 20 | 29 | 172 | 154 | 203 |
| | - | 3.38 | 27 | 39 | 208 | 170 | 284 |
| | - | 4.23 | 22 | 58 | 224 | 215 | 326 |
| | + | 0.85 | C | 10 | 28 | 26 | 151 |
| | + | 1.69 | 20 | 14 | 15 | 25 | 175 |
| | + | 2.54 | 13 | 11 | 42 | 41 | 169 |
| | + | 3.38 | 13 | 8 | 49 | 53 | 185 |
| | + | 4.23 | 24 | 15 | 51 | 64 | 214 |

Table A-38

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 34

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Reverants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 52 | 16 | 13 | 38 | 333 |
| | + | | 87 | 71 | 9 | 57 | 340 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1590 | | | | |
| 9-Aminoacridine | - | 100 | | 475 | | | |
| 2-Nitrofluorene | - | 50 | | | 2054 | | |
| AF2 | - | 0.1 | | | | 46 | 275 |
| 2-Anthramine | + | 20 | 516 | 760 | 2800 | 3000 | C* |
| Pre-ozonation | - | 9.8 | 36 | 45 | 163 | 217 | 355 |
| 7-50 LAP | + | 9.8 | 28 | 18 | 60 | 71 | 345 |
| Post-ozonation | - | 0.1 | 56 | 15 | 10 | 30 | 304 |
| 7-50 LAP | - | 0.3 | 57 | 13 | 11 | 41 | 307 |
| | - | 0.7 | 56 | 16 | 25 | 39 | 292 |
| | - | 1.4 | 54 | 22 | 33 | 48 | 304 |
| | - | 2.8 | 56 | 17 | 71 | 103 | 320 |
| | - | 7.1 | 46 | 26 | 145 | 185 | 328 |
| | + | 0.1 | 24 | 18 | 13 | 28 | 351 |
| | + | 0.3 | 27 | 12 | 21 | 39 | 383 |
| | + | 0.7 | 28 | 17 | 14 | 33 | 352 |
| | + | 1.4 | 32 | 14 | 23 | 43 | 326 |
| | + | 2.8 | 36 | 17 | 35 | 54 | 330 |
| | + | 7.1 | 25 | 14 | 64 | 74 | 353 |

*C, Contaminated.

Table A-39

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 37

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 7 | 10 | 11 | 14 | 92 |
| | + | | 5 | 7 | 9 | 19 | 77 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 262 | | | | |
| 9-Aminoacridine | - | 100 | | 1405 | | | |
| 2-Nitrofluorene | - | 50 | | | 1130 | | |
| AF2 | - | 0.1 | | | | 400 | 1176 |
| 2-Anthramine | + | 20 | 330 | 247 | 1825 | 1836 | 1290 |
| Pre-ozonation | - | 1.6 | 21 | 7 | 46 | 55 | 110 |
| 7-50 LAP | - | 3.2 | 9 | 22 | 80 | 85 | 120 |
| | - | 4.8 | 11 | 37 | 144 | 130 | 123 |
| | - | 6.4 | 16 | 43 | 204 | 205 | 152 |
| | - | 8 | 21 | 68 | 290 | 269 | 196 |
| | + | 1.6 | 10 | 3 | 4 | 21 | 104 |
| | + | 3.2 | 12 | 2 | 7 | 21 | 95 |
| | + | 4.8 | 7 | 5 | 8 | 15 | 89 |
| | + | 6.4 | 5 | 1 | 9 | 17 | 86 |
| | + | 8 | 7 | 3 | 10 | 17 | 102 |

Post-ozonation 7-50 LAP contaminated.

Table A-40

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-50 LAP

EXPERIMENT 44

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | | |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 494 | 1080 |
| | | | | | | 705 | 998 |
| Pre-ozonation | | | | | | | |
| 7-50 LAP | - | 7.9 | 21 | 38 | 185 | 295 | 340 |
| | + | 7.9 | 20 | 16 | 37 | 45 | 267 |
| Post-ozonation | | | | | | | |
| 7-50 LAP | - | 1.5 | 21 | 11 | 106 | 90 | 190 |
| | - | 3.1 | 18 | 37 | 144 | 146 | 237 |
| | - | 4.6 | 28 | 29 | 197 | 174 | 301 |
| | - | 6.2 | 24 | 46 | 200 | 202 | 333 |
| | - | 7.7 | 20 | 51 | 290 | 198 | 408 |
| | + | 1.5 | 12 | 5 | 15 | 29 | 201 |
| | + | 3.1 | 19 | 10 | 23 | 47 | 204 |
| | + | 4.6 | 14 | 12 | 30 | 37 | 253 |
| | + | 6.2 | 15 | 13 | 39 | 52 | 271 |
| | + | 7.7 | 27 | 16 | 61 | 59 | 255 |

Table A-41

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 20

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA98 |
|---|---|---|---|
| **Negative control** | - | | 52 |
| | + | | 52 |
| **Positive controls** | | | |
| **AF2** | - | 0.1 µg | 450 |
| **2-Anthramine** | + | 20 | 3000 |
| **Pre-chlorination** | - | 0.25 ml | 750 |
| 7-100 LAP | + | 0.25 | 70 |
| **Post-chlorination** | - | 0.005 ml | 58 |
| 7-100 LAP | - | 0.01 | 42 |
| | - | 0.025 | 98 |
| | - | 0.05 | 173 |
| | - | 0.1 | 368 |
| | - | 0.25 | 484 |
| | + | 0.005 | 42 |
| | + | 0.01 | 46 |
| | + | 0.025 | 42 |
| | + | 0.05 | 37 |
| | + | 0.1 | 65 |
| | + | 0.25 | 87 |

*100% Photolysis.

Table A-42

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 22

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 42 | 22 | 45 | 185 |
| | + | | 32 | 22 | 60 | 246 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 µg | 1830 | | | |
| 9-Aminoacridine | - | 100 | | 1500 | | |
| 2-Nitrofluorene | - | 50 | | | 1065 | |
| AF2 | - | 0.1 | | | | 3000 |
| 2-Anthramine | + | 20 | 2010 | 1515 | 6000 | 7000 |
| Pre-chlorination† | - | 0.005 ml | 72 | 11 | 25 | 286 |
| 7-100 LAP | - | 0.01 | 60 | 18 | 30 | 270 |
| | - | 0.025 | 62 | 15 | 41 | 295 |
| | - | 0.05 | 43 | 17 | 67 | 244 |
| | - | 0.1 | 36 | 13 | 79 | 262 |
| | - | 0.25 | 52 | 29 | 184 | 353 |
| | + | 0.005 | 36 | 18 | 47 | 254 |
| | + | 0.01 | 25 | 19 | 31 | 280 |
| | + | 0.025 | 28 | 18 | 36 | 250 |
| | + | 0.05 | 25 | 8 | 29 | 288 |
| | + | 0.1 | 27 | 20 | 40 | 314 |
| | + | 0.25 | 30 | 18 | 77 | 230 |

* 100% Photolysis.

† No post-chlorination was done.

Table A-43

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 44

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 µg | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Pre-chlorination | - | 0.05 ml | 29 | 30 | 149 | 131 | 150 |
| 7-100 LAP | - | 0.1 | 28 | 30 | 228 | 191 | 216 |
| | - | 0.15 | 20 | 38 | 306 | 294 | 217 |
| | - | 0.2 | 14 | 49 | 388 | 381 | 257 |
| | - | 0.25 | 25 | 63 | 526 | 425 | 288 |
| | + | 0.05 | 8 | 12 | 22 | 29 | 150 |
| | + | 0.1 | 14 | 14 | 37 | 36 | 147 |
| | + | 0.15 | 13 | 10 | 45 | 55 | 141 |
| | + | 0.2 | 13 | 14 | 64 | 63 | 162 |
| | + | 0.25 | 17 | 17 | 66 | 48 | 175 |

* 100% Photolized.

Table A-43 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 µg | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Post-chlorination | - | 0.05 ml | 25 | 13 | 94 | 86 | 167 |
| 7-100 LAP | - | 0.1 | 29 | 19 | 170 | 142 | 171 |
| | - | 0.15 | 18 | 25 | 238 | 189 | 181 |
| | - | 0.2 | 26 | 39 | 336 | 263 | 237 |
| | - | 0.25 | 15 | 66 | 375 | 305 | 233 |
| | + | 0.05 | 19 | 11 | 20 | 22 | 167 |
| | + | 0.1 | 11 | 9 | 29 | 43 | 155 |
| | + | 0.15 | 12 | 9 | 57 | 45 | 162 |
| | + | 0.2 | 13 | 9 | 41 | 48 | 127 |
| | + | 0.25 | 16 | 14 | 75 | 60 | 158 |

Table A-44

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 34

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 52 | 16 | 13 | 38 | 333 |
| | + | | 87 | 17 | 9 | 57 | 340 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1590 | | | | |
| 9-Aminoacridine | - | 100 | | 475 | | | |
| 2-Nitrofluorene | - | 50 | | | 2054 | | |
| AF2 | - | 0.1 | | | | 46 | 275 |
| 2-Anthramine | + | 20 | 516 | 760 | 2800 | 3000 | C† |
| Pre-ozonation | - | 0.25 ml | 26 | 68 | 316 | 300 | 323 |
| 7-100 LAP | + | 0.25 | 63 | 23 | 80 | 76 | 359 |
| Post-ozonation | - | 0.005 ml | 40 | 4 | 11 | 28 | 270 |
| 7-100 LAP | - | 0.01 | 66 | 11 | 18 | 32 | 256 |
| | - | 0.025 | 42 | 16 | 38 | 31 | 322 |
| | - | 0.05 | 49 | 19 | 60 | 78 | 300 |
| | - | 0.1 | 60 | 20 | 75 | 86 | 301 |
| | - | 0.25 | 52 | 50 | 152 | 220 | 323 |
| | + | 0.005 | 30 | 13 | 11 | 37 | 320 |
| | + | 0.01 | C | 12 | 16 | 37 | 329 |
| | + | 0.025 | 60 | 22 | 16 | 40 | 321 |
| | + | 0.05 | 60 | 13 | 32 | 37 | 325 |
| | + | 0.1 | 79 | 30 | 50 | 64 | 297 |
| | + | 0.25 | 90 | 34 | 95 | 83 | 282 |

* 100 Photolyzed.
† C, Contaminated.

Table A-45

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

7-100 LAP*

EXPERIMENT 40

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 9 | 19 | 115 |
| | + | | 9 | 14 | 23 | 28 | 119 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 456 | | | | |
| 9-Aminoacridine | - | 100 | | 546 | | | |
| 2-Nitrofluorene | - | 50 | | | 1295 | | |
| AF2 | - | 0.1 | | | | 98 | 446 |
| 2-Anthramine | + | 10 | 604 | 444 | 2560 | 2320 | 1755 |
| Pre-ozonation | - | 0.05 ml | 14 | 27 | 171 | 120 | 159 |
| 7-100 LAP | - | 0.1 | 27 | 29 | 283 | 209 | 204 |
| | - | 0.15 | 28 | 50 | 440 | 303 | 260 |
| | - | 0.2 | 23 | 66 | 483 | 354 | 292 |
| | - | 0.25 | 12 | 70 | 624 | 417 | 365 |
| | + | 0.05 | 14 | 13 | 28 | 38 | 127 |
| | + | 0.1 | 12 | 11 | 36 | 56 | 101 |
| | + | 0.15 | 27 | 6 | 66 | 50 | 93 |
| | + | 0.2 | 19 | 14 | 62 | 60 | 132 |
| | + | 0.25 | 11 | 8 | 107 | 79 | 170 |

* 100% Photolysis.

Table A-45 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Post-ozonation 7-100 LAP | - | 0.05 ml | 45 | 29 | 280 | 187 | 190 |
| | - | 0.1 | 68 | 71 | 491 | 297 | 279 |
| | - | 0.15 | 106 | 82 | 587 | 423 | 350 |
| | - | 0.2 | 105 | 83 | 950 | 493 | 357 |
| | - | 0.25 | 133 | 122 | 897 | 552 | 430 |
| | + | 0.05 | 35 | 19 | 48 | 46 | 143 |
| | + | 0.1 | 44 | 34 | 103 | 71 | 155 |
| | + | 0.15 | 56 | 40 | 122 | 88 | 155 |
| | + | 0.2 | 58 | 35 | 163 | 90 | 193 |
| | + | 0.25 | 56 | 104 | 288 | 120 | 231 |

Table A-46

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

9-100 LAP*

EXPERIMENT 22

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 42 | 22 | 45 | 185 |
| | + | | 32 | 22 | 60 | 246 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 µg | 1830 | | | |
| 9-Aminoacridine | - | 100 | | 1500 | | |
| 2-Nitrofluorene | - | 50 | | | 1065 | |
| AF2 | - | 0.1 | | | | 3000 |
| 2-Anthramine | + | 2.5 | 2010 | 1515 | 6000 | 7000 |
| Pre-chlorination | - | 0.005 ml | 55 | 20 | 33 | 271 |
| 9-100 LAP | - | 0.01 | 54 | 20 | 30 | 230 |
| | - | 0.025 | 67 | 16 | 38 | 250 |
| | - | 0.05 | 70 | 19 | 16 | 300 |
| | - | 0.1 | 61 | 22 | 33 | 276 |
| | - | 0.25 | 56 | 23 | 41 | 276 |
| | + | 0.005 | 21 | 15 | 24 | 334 |
| | + | 0.01 | 31 | 21 | 25 | 250 |
| | + | 0.025 | 36 | 34 | 30 | 300 |
| | + | 0.05 | 16 | 17 | 32 | 450 |
| | + | 0.1 | 35 | 22 | 32 | 270 |
| | + | 0.25 | 23 | 19 | 46 | 270 |

* Solution photolyzed.

Table A-47

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

9-100 LAP*

EXPERIMENT 44

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 µg | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Pre-chlorination 9-100 LAP | - | 0.05 ml | 20 | 11 | 34 | 38 | 208 |
| | - | 0.1 | 38 | 19 | 44 | 40 | 278 |
| | - | 0.15 | 35 | 18 | 83 | 61 | 304 |
| | - | 0.2 | 28 | 36 | 83 | 89 | 291 |
| | - | 0.25 | 22 | 27 | 102 | 92 | 429 |
| | + | 0.05 | 16 | 15 | 15 | 17 | 182 |
| | + | 0.1 | 16 | 8 | 26 | 30 | 170 |
| | + | 0.15 | 14 | 21 | 31 | 28 | 189 |
| | + | 0.2 | 19 | 15 | 30 | 47 | 244 |
| | + | 0.25 | 20 | 16 | 36 | 33 | 255 |

* Solution photolyzed.

Table A-47 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 µg | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Post-chlorination | - | 0.05 ml | 22 | 16 | 19 | 28 | 185 |
| 9-100 LAP | - | 0.1 | 27 | 8 | 31 | 42 | 287 |
| | - | 0.15 | 31 | 24 | 42 | 47 | 332 |
| | - | 0.2 | 27 | 20 | 55 | 71 | 355 |
| | - | 0.25 | 25 | 28 | 70 | 93 | 403 |
| | + | 0.05 | 18 | 5 | 13 | 31 | 175 |
| | + | 0.1 | 18 | 9 | 25 | 37 | 211 |
| | + | 0.15 | 15 | 15 | 29 | 27 | 294 |
| | + | 0.2 | 17 | 12 | 39 | 43 | 254 |
| | + | 0.25 | 27 | 15 | 55 | 35 | 302 |

Table A-48

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,6-DINITROTOLUENE

EXPERIMENT 23

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 29 | 15 | 34 | 49 | 164 |
| | + | | 17 | 24 | 38 | 33 | 187 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1100 | | | | |
| 9-Aminoacridine | - | 100 | | 500 | | | |
| 2-Nitrofluorene | - | 50 | | | 850 | | |
| AF2 | - | 0.1 | | | | 74 | 650 |
| 2-Anthramine | + | 20 | 570 | 860 | ~1300 | ~1100 | ~1400 |
| Pre-chlorination | - | 45 | 31 | 22 | 22 | 33 | 201 |
| 2,6-Dinitrotoluene | + | 45 | 26 | 13 | 31 | 46 | 161 |
| Post-chlorination | - | 0.9 | 31 | 21 | 13 | 37 | 193 |
| 2,6-Dinitrotoluene | - | 1.8 | 36 | 32 | C* | 36 | 181 |
| | - | 4.5 | 45 | 19 | 27 | 41 | 211 |
| | - | 9 | 38 | 21 | 31 | 29 | 192 |
| | - | 18 | 34 | 17 | C | C | C |
| | - | 45 | C | C | C | C | C |
| | + | 0.9 | 19 | 22 | 32 | 52 | 177 |
| | + | 1.8 | 21 | 13 | 43 | 46 | 209 |
| | + | 4.5 | 23 | 19 | 35 | 53 | 151 |
| | + | 9 | 29 | 15 | 31 | 58 | 182 |
| | + | 18 | 24 | 12 | C | C | C |
| | + | 45 | C | C | C | C | C |

* C, Contaminated.

Table A-49

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,6-DINITROTOLUENE

EXPERIMENT 31

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 17 | 11 | 14 | 14 | 164 |
| | + | | 8 | 9 | 15 | 20 | 147 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 650 | | | | |
| 9-Aminoacridine | - | 100 | | 250 | | | |
| 2-Nitrofluorene | - | 50 | | | 291 | | |
| AF2 | - | 0.1 | | | | 21 | 560 |
| 2-Anthramine | + | 20 | 10 | 430 | 1600 | 94 | 1500 |
| Pre-chlorination | | | | | | | |
| 2,6-Dinitrotoluene | - | 15 | 23 | 10 | 16 | 12 | 195 |
| | + | 15 | 8 | 6 | 11 | 16 | 171 |
| Post-chlorination | | | | | | | |
| 2,6-Dinitrotoluene | - | 0.3 | 24 | 11 | 7 | 24 | 176 |
| | - | 0.6 | 27 | 15 | 4 | 12 | 167 |
| | - | 1.5 | 22 | 20 | 11 | 32 | 186 |
| | - | 3 | 57 | 38 | 30 | C* | 233 |
| | - | 6 | 84 | 64 | 65 | 107 | 235 |
| | - | 15 | 204 | 147 | 230 | 208 | 418 |
| | + | 0.3 | 17 | 11 | 15 | 24 | 200 |
| | + | 0.6 | 18 | 14 | 33 | 18 | 200 |
| | + | 1.5 | 28 | 25 | 26 | 32 | 201 |
| | + | 3 | 47 | 24 | 50 | 47 | 250 |
| | + | 6 | 47 | 60 | 64 | 70 | 271 |
| | + | 15 | 111 | 130 | 110 | 170 | 369 |

* C, Contaminated.

Table A-50

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,6-DINITROTOLUNE

EXPERIMENT 44

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 13 | 4 | 6 | 22 | 133 |
| | + | | 9 | 5 | 11 | 26 | 139 |
| Positive controls | | | | | | | |
| Sodium azide | - | 1 | 238 | | | | |
| 9-Aminoacridine | - | 100 | | 1152 | | | |
| 2-Nitrofluorene | - | 50 | | | 1608 | | |
| AF2 | - | 0.1 | | | | 494 | 1080 |
| 2-Anthramine | + | 2.5 | 104 | 67 | 726 | 705 | 998 |
| Pre-chlorination | - | 0.6 | 16 | 7 | 9 | 26 | 127 |
| 2,6-Dinitrotoluene | + | 0.6 | 16 | 5 | 17 | 23 | 166 |
| Post-chlorination | - | 0.12 | 15 | 4 | 8 | 13 | 140 |
| 2,6-Dinitrotoluene | - | 0.24 | 23 | 6 | 7 | 12 | 128 |
| | - | 0.36 | 20 | 9 | 3 | 18 | 134 |
| | - | 0.48 | 22 | 6 | 6 | 16 | 124 |
| | - | 0.6 | 24 | 4 | 12 | 15 | 115 |
| | + | 0.12 | 13 | 4 | 14 | 17 | 137 |
| | + | 0.24 | 14 | 10 | 14 | 15 | 124 |
| | + | 0.36 | 23 | 6 | 18 | 22 | 169 |
| | + | 0.48 | 26 | 3 | 18 | 22 | 153 |
| | + | 0.6 | 26 | 7 | 26 | 19 | 155 |

Table A-51

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,6-DINITROTOLUENE

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 28 | 23 | 29 | 83 |
| | + | | 12 | 40 | 21 | 44 | 108 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 550 | | | | |
| 9-Aminoacridine | - | 100 | | 430 | | | |
| 2-Nitrofluorene | - | 50 | | | 65 | | |
| AF2 | - | 0.1 | | | | 257 | 324 |
| 2-Anthramine | + | 20 | 332 | 850 | 1800 | 1200 | 1900 |
| Pre-ozonation | - | 5 | 23 | 23 | 22 | 23 | 93 |
| 2,6-Dinitrotoluene | + | 5 | 14 | 22 | 34 | 59 | 94 |
| Post-ozonation | - | 0.04 | 21 | 21 | 13 | 30 | 81 |
| 2,6-Dinitrotoluene | - | 0.08 | 20 | 19 | 13 | 33 | 93 |
| | - | 0.2 | 26 | 33 | 21 | 25 | 90 |
| | - | 0.4 | 38 | 26 | 14 | 26 | 79 |
| | - | 0.8 | 36 | 18 | 13 | 29 | 75 |
| | - | 2 | 37 | 24 | 15 | 33 | 102 |
| | + | 0.04 | 11 | 34 | 18 | 31 | 102 |
| | + | 0.08 | 20 | 27 | 26 | 37 | 96 |
| | + | 0.2 | 20 | 21 | 28 | 24 | 118 |
| | + | 0.4 | 15 | 27 | 23 | 43 | 103 |
| | + | 0.8 | 17 | 29 | 22 | 38 | 95 |
| | + | 2 | 16 | 25 | 27 | 52 | 119 |

Table A-52

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,6-DINITROTOLUENE

EXPERIMENT 34

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | - | | 52 | 16 | 13 | 38 | 333 |
| | + | | 87 | 17 | 9 | 57 | 340 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1590 | | | | |
| 9-Aminoacridine | - | 100 | | 475 | | | |
| 2-Nitrofluorene | - | 50 | | | 2054 | | |
| AF2 | - | 0.1 | | | | 46 | 275 |
| 2-Anthramine | + | 20 | 516 | 760 | 2800 | 3000 | C* |
| Pre-Ozonation | - | 35 | 64 | 12 | 9 | 33 | 320 |
| 2,6-Dinitrotoluene | + | 35 | C | 12 | 14 | 35 | 340 |
| Post-Ozonation | - | 5.6 | 73 | 8 | 11 | 33 | 352 |
| 2,6-Dinitrotoluene | - | 11.2 | 57 | 15 | 11 | 29 | 332 |
| | - | 16.8 | 64 | 4 | 13 | 27 | 293 |
| | - | 22.4 | 55 | 13 | 15 | 32 | 286 |
| | - | 28 | 87 | 12 | 9 | 32 | 335 |
| | + | 5.6 | 52 | 20 | 8 | 32 | 335 |
| | + | 11.2 | 65 | 18 | 23 | 32 | 302 |
| | + | 16.8 | 102 | 13 | 25 | 38 | 366 |
| | + | 22.4 | 66 | 13 | 14 | 40 | 346 |
| | + | 28 | 70 | 6 | 26 | 28 | 368 |

* C, Contaminated.

Table A-53

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4-DINITROTOLUENE

EXPERIMENT 18

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 72 | 28 | 34 | 78 | 116 |
| | + | | 24 | 54 | 41 | 90 | 129 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | ~800 | | | | |
| 9-Aminoacridine | - | 100 | | C* | | | |
| 2-Nitrofluorene | - | 50 | | | C | | |
| AF2 | - | 0.1 | | | | ~ 600 | ~1500 |
| 2-Anthramine | + | 20 | ~600 | ~550 | ~1500 | ~1000 | ~2000 |
| Pre-chlorination | - | 53 | 61 | 34 | 28 | 72 | 125 |
| 2,4-Dinitrotoluene | + | 53 | C | 75 | C | 117 | C |
| Post-chlorination | - | 1 | 88 | 37 | 32 | 83 | 120 |
| 2,4-Dinitrotoluene | - | 2 | 63 | 24 | 27 | 75 | 137 |
| | - | 5 | 57 | 27 | 24 | 97 | 106 |
| | - | 10 | 74 | C | 36 | 89 | 161 |
| | - | 20 | 57 | C | C | C | 166 |
| | - | 50 | C | C | C | C | C |
| | + | 1 | C | 70 | 44 | 112 | 119 |
| | + | 2 | C | 96 | 45 | 86 | 99 |
| | + | 5 | C | C | 43 | 103 | 106 |
| | + | 10 | C | C | 37 | 106 | 103 |
| | + | 20 | C | C | C | 135 | 162 |
| | + | 50 | C | C | C | C | 158 |
| | | | | | 43 | C | 119 |

* C, Contaminated.

Table A-54

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4-DINITROTOLUENE

EXPERIMENT 28

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 9 | 85 | 117 |
| | + | | 21 | 10 | 22 | 74 | 121 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 2400 | | | | |
| 9-Aminoacridine | - | 100 | | 1000 | | | |
| 2-Nitrofluorene | - | 50 | | | 1500 | | |
| AF2 | - | 0.1 | | | | 260 | 500 |
| 2-Anthramine | + | 20 | 336 | 81 | 270 | 1200 | 2000 |
| Pre-chlorination | - | 35 | 24 | 19 | 21 | 91 | 132 |
| 2,4-Dinitrotoluene | + | 35 | 25 | 18 | 27 | 102 | 174 |
| Post-chlorination | - | 0.67 | 38 | 14 | 12 | 102 | 180 |
| 2,4-Dinitrotoluene | - | 1.34 | 27 | 8 | 24 | 96 | 132 |
| | - | 3.35 | 31 | 17 | 21 | 90 | 136 |
| | - | 6.7 | 16 | 15 | 15 | 94 | 141 |
| | - | 13.4 | 13 | 17 | 14 | 68 | 157 |
| | - | 33.5 | 15 | 11 | 16 | 93 | 160 |
| | + | 0.67 | 35 | 18 | 17 | 108 | 179 |
| | + | 1.34 | 32 | 15 | 13 | 87 | 129 |
| | + | 3.35 | 45 | 12 | 16 | 107 | 114 |
| | + | 6.7 | 25 | 8 | 23 | 80 | 135 |
| | + | 13.4 | 25 | 9 | 19 | 91 | 148 |
| | + | 33.5 | 31 | 18 | 18 | 80 | 133 |

Table A-55

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4-DINITROTOLUENE

EXPERIMENT 39

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 67 | 11 | 22 | 32 | 92 |
| | + | | 102 | 13 | 16 | 29 | 114 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 157 | | | | |
| 9-Aminoacridine | - | 100 | | 1252 | | | |
| 2-Nitrofluorene | - | 50 | | | 9 | | |
| AF2 | - | 0.1 | | | | 218 | 554 |
| 2-Anthramine | + | | 381 | 79 | 777 | 1632 | 2138 |
| Pre-chlorination 2,4-Dinitrotoluene | - | 1.2 | 104 | 11 | 9 | 38 | 115 |
| | - | 3.1 | 80 | 13 | 16 | 29 | 89 |
| | - | 6.2 | 104 | 14 | 10 | 24 | 84 |
| | - | 12.3 | 90 | 4 | 8 | 23 | 75 |
| | - | 30.8 | 82 | 5 | 11 | 25 | 97 |
| | + | 1.2 | 65 | 11 | 17 | 35 | 110 |
| | + | 3.1 | 77 | 18 | 19 | 34 | 86 |
| | + | 6.2 | 78 | 12 | 16 | 27 | 70 |
| | + | 12.3 | 86 | 14 | 25 | 38 | 76 |
| | + | 30.8 | 65 | 17 | 17 | 40 | 83 |

Table A-55 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Post-chlorination 2,4-Dinitrotoluene | - | 1.2 | 89 | 9 | 8 | 30 | 101 |
| | - | 3.1 | 83 | 7 | 2 | 22 | 99 |
| | - | 6.2 | 95 | 5 | 8 | 21 | 110 |
| | - | 12.3 | 85 | 13 | 6 | 29 | 111 |
| | - | 30.8 | 93 | 8 | 11 | 19 | 96 |
| | + | 1.2 | 100 | 9 | 13 | 29 | 103 |
| | + | 3.1 | 119 | 12 | 18 | 27 | 91 |
| | + | 6.2 | 103 | 11 | 17 | 35 | 83 |
| | + | 12.3 | 118 | 19 | 14 | 46 | 100 |
| | + | 30.8 | 106 | 10 | 17 | 39 | 102 |

Table A-56

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

3,5-DINITROTOLUENE

EXPERIMENT 17

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 42 | 23 | 15 | 125 | 166 |
| | + | | 29 | 28 | 41 | 174 | 163 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | ~1700 | | | | |
| 9-Aminoacridine | - | 100 | | 50 | | | |
| 2-Nitrofluorene | - | 50 | | | ~3500 | | |
| AF2 | - | 0.1 | | | | 591 | ~1750 |
| 2-Anthramine | + | 20 | ~ 720 | 348 | ~7500 | ~800 | >10000 |
| Pre-chlorination | - | 32 | 42 | 15 | 26 | 113 | 127 |
| 3,5-Dinitrotoluene | + | 32 | 21 | 19 | 26 | 125 | 99 |
| Post-chlorination | - | 0.61 | 61 | 17 | 18 | 120 | 163 |
| 3,5-Dinitrotoluene | - | 1.22 | 47 | 12 | 11 | 182 | 145 |
| | - | 3.05 | 49 | 16 | 20 | 118 | 176 |
| | - | 6.1 | 50 | 13 | 21 | 129 | 166 |
| | - | 12.2 | 49 | 29 | 19 | 117 | 153 |
| | - | 30.5 | 48 | 12 | 30 | 121 | 184 |
| | + | 0.61 | 24 | 31 | 30 | 104 | 181 |
| | + | 1.22 | 16 | 28 | 23 | 141 | 169 |
| | + | 3.05 | 21 | 23 | 25 | 135 | 159 |
| | + | 6.1 | 20 | 29 | 22 | 119 | 156 |
| | + | 12.2 | 20 | 23 | 21 | 101 | 87 |
| | + | 30.5 | 23 | 30 | 27 | 134 | 138 |

Table A-57

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

3,5-DINITROTOLUENE

EXPERIMENT 18

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate | | | | |
|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 |
| Negative control | - | | 72 | 28 | 34 | 78 | 116 |
| | + | | 24 | 54 | 41 | 90 | 129 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 800 | | | | |
| 9-Aminoacridine | - | 100 | | C* | | | |
| 2-Nitrofluorene | - | 50 | | | C | | |
| AF2 | - | 0.1 | | | | ~ 600 | ~1500 |
| 2-Anthramine | + | 20 | ~600 | ~550 | ~1500 | ~1000 | ~2000 |
| Pre-chlorination | - | 4.2 | 86 | 46 | 31 | 97 | 105 |
| 3,5-Dinitrotoluene | + | 4.2 | 32 | 70 | 27 | 92 | 102 |
| Post-chlorination | - | 0.08 | 84 | 36 | 31 | 109 | 107 |
| 3,5-Dinitrotoluene | - | 0.16 | 105 | 36 | 47 | 82 | 106 |
| | - | 0.4 | 93 | 39 | 27 | 85 | 127 |
| | - | 0.8 | 121 | 35 | 24 | 65 | 113 |
| | - | 1.6 | 110 | 36 | 31 | C | 107 |
| | - | 4 | 87 | 42 | 25 | 70 | 98 |
| | + | 0.08 | 32 | 96 | 49 | 90 | 126 |
| | + | 0.16 | 34 | 69 | 52 | 100 | 119 |
| | + | 0.4 | 29 | 80 | C | 97 | 130 |
| | + | 0.8 | 40 | 65 | 41 | 83 | 112 |
| | + | 1.6 | 28 | 71 | C | 73 | 121 |
| | + | 4 | 37 | 59 | C | 96 | 106 |

* C, Contaminated.

Table A-58

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROTOLUENE

EXPERIMENT 18

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 72 | 28 | 34 | 78 | 116 |
| | + | | 24 | 54 | 41 | 90 | 129 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | ~800 | | | | |
| 9-Aminoacridine | - | 100 | | C* | | | |
| 2-Nitrofluorene | - | 50 | | | C | | |
| AF2 | - | 0.1 | | | | ~600 | ~1500 |
| 2-Anthramine | + | 20 | ~600 | ~550 | ~1500 | ~1000 | ~2000 |
| Pre-chlorination | - | 35 | 56 | 26 | 25 | 102 | 118 |
| 2,4,6-Trinitrotoluene | + | 35 | 29 | 54 | 23 | 73 | 153 |
| Post-chlorination | - | 0.67 | 68 | 23 | 27 | 79 | 107 |
| 2,4,6-Trinitrotoluene | - | 1.34 | 82 | 22 | 37 | 90 | 177 |
| | - | 3.35 | 75 | 23 | 31 | 63 | 141 |
| | - | 6.7 | 57 | 23 | 28 | 89 | 154 |
| | - | 13.4 | 75 | 28 | 33 | 97 | 124 |
| | - | 33.5 | 47 | 24 | 34 | 108 | 182 |
| | + | 0.67 | 25 | 61 | 38 | 83 | 117 |
| | + | 1.34 | 27 | 39 | 33 | 79 | 117 |
| | + | 3.35 | 22 | 46 | 24 | 77 | 122 |
| | + | 6.7 | 31 | 54 | 32 | 94 | 143 |
| | + | 13.4 | 27 | 59 | 39 | 74 | 161 |
| | + | 33.5 | 22 | 57 | 32 | 71 | 175 |

* C, Contaminated.

Table A-59

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROTOLUENE

EXPERIMENT 28

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 9 | 85 | 117 |
| | + | | 21 | 10 | 22 | 74 | 121 |
| Positive controls | | | | | | | |
| ß-Propiolactone | - | 50 | 2400 | | | | |
| 9-Aminoacridine | - | 100 | | 1000 | | | |
| 2-Nitrofluorene | - | 50 | | | 1500 | | |
| AF2 | - | 0.1 | | | | 260 | 500 |
| 2-Anthramine | + | 20 | 336 | 81 | 270 | 1200 | 2000 |
| Pre-chlorination | - | 21 | 32 | 17 | 30 | 66 | 161 |
| 2,4,6-Trinitrotoluene | + | 21 | 29 | 15 | 18 | 75 | 172 |
| Post-chlorination | - | 0.4 | 25 | 11 | 20 | 75 | 145 |
| 2,4,6-Trinitrotoluene | - | 0.8 | 17 | 6 | 22 | 99 | 130 |
| | - | 2 | 46 | 10 | 18 | 84 | 147 |
| | - | 4 | 23 | 22 | 25 | 74 | 162 |
| | - | 8 | 38 | 17 | 18 | 84 | 155 |
| | - | 20 | 34 | 16 | 22 | 82 | 178 |
| | + | 0.4 | 38 | 16 | 22 | 90 | 177 |
| | + | 0.8 | 25 | 15 | 25 | 93 | 171 |
| | + | 2 | 26 | 21 | 18 | 78 | 157 |
| | + | 4 | 32 | 21 | 23 | 82 | 178 |
| | + | 8 | 35 | 20 | 24 | 88 | 199 |
| | + | 20 | 33 | 27 | 13 | 71 | 162 |

Table A-60

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROTOLUENE

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 28 | 23 | 29 | 83 |
| | + | | 12 | 40 | 21 | 44 | 108 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 550 | | | | |
| 9-Aminoacridine | - | 100 | | 430 | | | |
| 2-Nitrofluorene | - | 50 | | | 65 | | |
| AF2 | - | 0.1 | | | | 257 | 324 |
| 2-Anthramine | + | 20 | 332 | 850 | 1800 | 1200 | 1900 |
| Pre-ozonation | - | 7.5 | 33 | 27 | 15 | 35 | 103 |
| 2,4,6-Trinitrotoluene | + | 7.5 | 17 | 17 | 29 | 42 | 131 |
| Post-ozonation | - | 0.13 | 46 | 26 | 19 | 24 | 97 |
| 2,4,6-Trinitrotoluene | - | 0.26 | 41 | 24 | 18 | 31 | 92 |
| | - | 0.65 | 34 | 21 | 13 | C* | 105 |
| | - | 1.3 | 44 | 26 | 21 | 46 | 89 |
| | - | 2.6 | 35 | 17 | 20 | 33 | 112 |
| | - | 6.5 | 43 | 33 | 19 | 28 | 91 |
| | + | 0.13 | 23 | 24 | 17 | 39 | 87 |
| | + | 0.26 | 9 | 33 | 24 | 29 | 165 |
| | + | 0.65 | 20 | 38 | 31 | 54 | 109 |
| | + | 1.3 | 15 | 29 | 30 | 43 | 123 |
| | + | 2.6 | 25 | 22 | 24 | 53 | 113 |
| | + | 6.5 | 18 | 27 | 35 | 44 | 126 |

* C, Contaminated.

Table A-61

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROTOLUENE

EXPERIMENT 36

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | - | | 28 | 5 | 9 | 22 | C* |
| | + | | 14 | 2 | 15 | 21 | C |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 224 | | | | C |
| 9-Aminoacridine | - | 100 | | 900 | | | C |
| 2-Nitrofluorene | - | 50 | | | 1300 | | C |
| AF2 | - | 0.1 | | | | 451 | C |
| 2-Anthramine | + | 20 | 416 | 322 | 1955 | 2200 | C |
| Pre-ozonation | - | 9.3 | 11 | 1 | 17 | 21 | C |
| 2,4,6-Trinitrotoluene | + | 9.3 | 5 | 3 | 17 | 19 | C |
| Post-ozonation | - | 1.6 | 29 | 4 | 11 | 21 | C |
| 2,4,6-Trinitrotoluene | - | 3.2 | 23 | 6 | 9 | 28 | C |
| | - | 4.8 | 23 | 7 | 6 | 22 | C |
| | - | 6.4 | 18 | 8 | 12 | 17 | C |
| | - | 8 | 22 | 6 | 12 | 29 | C |
| | + | 1.6 | 12 | 4 | 10 | 14 | C |
| | + | 3.2 | 11 | 6 | 6 | 16 | C |
| | + | 4.8 | 10 | 5 | 7 | 16 | C |
| | + | 6.4 | 10 | 6 | 6 | 9 | C |
| | + | 8 | 6 | 6 | 7 | 26 | C |

* C, Contaminated.

Table A-62

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROTOLUENE

EXPERIMENT 40

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative control | - | | 115 |
| | + | | 119 |
| Positive controls | | | |
| AF2 | - | 0.1 | 446 |
| 2-Anthramine | + | 10.1 | 1755 |
| Pre-ozonation | - | 8.9 | 150 |
| 2,4,6-Trinitrotoluene | + | 8.9 | 154 |
| Post-ozonation | - | 1.5 | 163 |
| 2,4,6-Trinitrotoluene | - | 3.0 | 164 |
| | - | 4.5 | 186 |
| | - | 6.0 | 151 |
| | - | 7.5 | 153 |
| | + | 1.5 | 130 |
| | + | 3.0 | 173 |
| | + | 4.5 | 181 |
| | + | 6.0 | 174 |
| | + | 7.5 | 194 |

Table A-63

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITRORESORCINOL

EXPERIMENT 17

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 42 | 23 | 15 | 125 | 166 |
| | + | | 29 | 28 | 41 | 174 | 163 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | ~1700 | | | | |
| 9-Aminoacridine | - | 100 | | 50 | | | |
| 2-Nitrofluorene | - | 50 | | | ~3500 | | |
| AF-2 | - | 0.1 | | | | 591 | ~1750 |
| 2-Anthramine | + | 20 | ~720 | 348 | ~7500 | ~800 | >10,000 |
| Pre-chlorination | - | 191 | 45 | 24 | 26 | 112 | 165 |
| 2,4,6-Trinitroresorcinol | | 191 | 34 | 15 | 20 | 121 | 161 |
| Post-chlorination | - | 3.6 | 54 | 15 | 14 | 141 | 153 |
| 2,4,6-Trinitroresorcinol | - | 7.2 | 58 | 23 | 15 | 117 | 158 |
| | - | 18 | 66 | 17 | 13 | 114 | 166 |
| | - | 36 | 71 | 19 | 15 | 119 | 183 |
| | - | 72 | 67 | 15 | 16 | 115 | 188 |
| | - | 180 | 47 | 16 | 23 | 116 | 161 |
| | + | 3.6 | 25 | 15 | 31 | 122 | 141 |
| | + | 7.2 | 18 | 16 | 33 | 121 | 143 |
| | + | 18 | 24 | 22 | 19 | 167 | 147 |
| | + | 36 | 28 | 19 | 29 | 108 | 156 |
| | + | 72 | 28 | 20 | 30 | 119 | 144 |
| | + | 180 | 36 | 19 | 27 | 134 | 161 |

Table A-64

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITRORESORCINOL

EXPERIMENT 28

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 9 | 85 | 117 |
| | + | | 21 | 10 | 22 | 74 | 121 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 2400 | | | | |
| 9-Aminoacridine | - | 100 | | 1000 | | | |
| 2-Nitrofluorene | - | 50 | | | 1500 | | |
| AF-2 | - | 0.1 | | | | 260 | 500 |
| 2-Anthramine | + | 20 | 336 | 81 | 270 | 1200 | 2000 |
| Pre-chlorination | - | 130 | 56 | 13 | 8 | 83 | 151 |
| 2,4,6-Trinitroresorcinol | + | 130 | 22 | 16 | 24 | 50 | 155 |
| Post-chlorination | - | 2.5 | 32 | 26 | 17 | 91 | 144 |
| 2,4,6-Trinitroresorcinol | - | 5 | 38 | 9 | 16 | 68 | 126 |
| | - | 12.5 | 19 | 14 | 14 | 100 | 150 |
| | - | 25 | 39 | 15 | 16 | 70 | 119 |
| | - | 50 | 35 | 9 | 18 | 60 | 176 |
| | - | 125 | 51 | 11 | 16 | 81 | 146 |
| | + | 2.5 | 21 | 21 | 18 | 68 | 144 |
| | + | 5 | 33 | 20 | 18 | 48 | 136 |
| | + | 12.5 | 25 | 11 | 19 | 53 | 163 |
| | + | 25 | 20 | 13 | 18 | 38 | 170 |
| | + | 50 | 17 | 9 | 16 | 45 | 170 |
| | + | 125 | 30 | 15 | 21 | 80 | 179 |

Table A-65

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITRORESORCINOL

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 34 | 7 | 12 | 14 | 82 |
| | + | | 14 | 6 | 24 | 25 | 96 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 547 | | | | |
| 9-Aminoacridine | - | 100 | | 400 | | | |
| 2-Nitrofluorene | - | 50 | | | 700 | | |
| AF-2 | - | 0.1 | | | | 200 | 300 |
| 2-Anthramine | + | 20 | 108 | 11 | 54 | 300 | 1200 |
| Pre-ozonation 2,4,6-Trinitroresorcinol | - | 218.8 | 34 | 6 | 18 | 38 | 84 |
| | + | 218.8 | 15 | 12 | 27 | C* | C |
| Post-ozonation 2,4,6-Trinitroresorcinol | - | 4.3 | 39 | 9 | 16 | 40 | 74 |
| | - | 8.6 | 36 | 4 | 30 | 68 | 84 |
| | - | 21.5 | 35 | 5 | 18 | 24 | 86 |
| | - | 42.9 | 29 | 11 | 23 | 23 | 85 |
| | - | 85.8 | 34 | 13 | 23 | 22 | 72 |
| | - | 214.4 | 33 | 28 | 37 | 20 | 91 |
| | + | 4.3 | 22 | 9 | 37 | 38 | C |
| | + | 8.6 | 21 | 8 | 34 | 61 | C |
| | + | 21.5 | 21 | 12 | 19 | 37 | C |
| | + | 42.9 | 30 | 11 | 39 | 25 | C |
| | + | 85.8 | 22 | 9 | 39 | 41 | C |
| | + | 214.4 | 27 | 23 | 25 | 40 | C |

* C, Contaminated.

Table A-66

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITRORESORCINOL

EXPERIMENT 35

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 30 | 8 | 13 | 22 | 73 |
| | + | | 18 | 12 | 19 | 39 | 71 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 1050 | | | | |
| 9-Aminoacridine | - | 100 | | 218 | | | |
| 2-Nitrofluorene | - | 50 | | | 2000 | | |
| AF2 | - | 0.1 | | | | 24 | 73 |
| 2-Anthramine | + | 20 | 500 | 1270 | 2300 | 2540 | 1875 |
| Pre-ozonation | - | 77 | 30 | 13 | 12 | 19 | 53 |
| 2,4,6-Trinitroresorcinol | + | 77 | 22 | 10 | 21 | 33 | C* |
| Post-ozonation | - | 14 | 49 | 10 | 24 | 28 | 75 |
| 2,4,6-Trinitroresorcinol | - | 28 | 52 | 18 | 25 | 38 | 92 |
| | - | 42 | 43 | 26 | 28 | 44 | 76 |
| | - | 56 | 42 | 46 | 27 | 40 | 85 |
| | - | 70 | 57 | 43 | 38 | 46 | 78 |
| | + | 14 | 60 | 40 | 60 | 33 | 79 |
| | + | 28 | 23 | 22 | 50 | 40 | 95 |
| | + | 42 | 45 | C | 52 | 46 | 83 |
| | + | 56 | 29 | 40 | 45 | 65 | 101 |
| | + | 70 | 40 | 40 | 55 | 49 | 97 |

* C, Contaminated.

Table A-67

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITRORESORCINOL

EXPERIMENT 40

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative control | - | | 115 |
| | + | | 119 |
| Positive controls | | | |
| AF2 | - | 0.1 | 446 |
| 2-Anthramine | + | 10 | 1755 |
| Pre-ozonation | - | 219 | 120 |
| 2,4,6-Trinitroresorcinol | + | 219 | 106 |
| Post-ozonation | - | 4 | 137 |
| 2,4,6-Trinitroresorcinol | - | 9 | 130 |
| | - | 21 | 144 |
| | - | 43 | 139 |
| | - | 86 | 120 |
| | - | 214 | 148 |
| | + | 4 | 105 |
| | + | 9 | 117 |
| | + | 21 | 100 |
| | + | 43 | 116 |
| | + | 86 | 116 |
| | + | 214 | 123 |

Table A-68

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 50 | 20 | 41 | 28 | 144 |
| | + | | 19 | 35 | 43 | 54 | 137 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1500 | | | | |
| 2-Nitrofluorene | - | 50 | | | 324 | | |
| 9-Aminoacridine | - | 100 | | 440 | | | |
| AF2 | - | 0.1 | | | | 600 | 1500 |
| 2-Anthramine | + | 20 | 217 | 500 | 3000 | 1000 | 6000 |
| Pre-chlorination 2,4,6-Trinitrobenzonitrile* | - | 0.25 ml | 61 | 11 | 22 | 95 | 218 |
| | + | 0.25 | 29 | 24 | 34 | 106 | 310 |
| Post-chlorination 2,4,6-Trinitrobenzonitrile* | - | 0.005 ml | 48 | 17 | 27 | 33 | 109 |
| | - | 0.01 | 51 | 20 | 25 | 41 | 144 |
| | - | 0.025 | 57 | 23 | 27 | 30 | 130 |
| | - | 0.05 | 46 | 15 | 24 | 46 | 120 |
| | - | 0.1 | 38 | 21 | 23 | 50 | 168 |
| | - | 0.25 | 51 | 18 | 21 | 51 | 180 |
| | + | 0.005 | 15 | 33 | 46 | 43 | 142 |
| | + | 0.01 | 15 | 42 | 22 | 45 | 132 |
| | + | 0.025 | 20 | 23 | 28 | 41 | 134 |
| | + | 0.05 | 19 | 19 | 33 | 38 | 146 |
| | + | 0.1 | 27 | 38 | 31 | 51 | 174 |
| | + | 0.25 | 15 | 41 | 31 | 58 | 194 |

* 100% Decomposition to picric acid and other products.

Table A-69

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 31

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 17 | 11 | 14 | 14 | 164 |
| | + | | 8 | 9 | 15 | 20 | 147 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 650 | | | | |
| 9-Aminoacridine | - | 100 | | 250 | | | |
| 2-Nitrofluorene | - | 50 | | | 291 | | |
| AF2 | - | 0.1 | | | | 21 | 560 |
| 2-Anthramine | + | 20 | 10 | 430 | 1600 | 94 | 1500 |
| Pre-chlorination | - | 0.25 ml | 41 | 12 | 18 | 22 | 780 |
| 2,4,6-Trinitrobenzonitrile | + | 0.25 | 17 | 13 | 23 | 18 | 585 |
| Post-chlorination | - | 0.05 ml | 33 | 5 | 18 | 23 | 249 |
| 2,4,6-Trinitrobenzonitrile | - | 0.1 | 37 | 8 | 20 | 17 | 348 |
| | - | 0.15 | 25 | 6 | 14 | 23 | 430 |
| | - | 0.2 | 54 | 9 | 9 | 34 | 496 |
| | - | 0.25 | 34 | 6 | 20 | 20 | 557 |
| | + | 0.05 | 14 | 10 | 19 | 18 | 236 |
| | + | 0.1 | 13 | 12 | 19 | 20 | 365 |
| | + | 0.15 | 28 | 12 | 17 | 25 | 415 |
| | + | 0.2 | 17 | 9 | 20 | 15 | 515 |
| | + | 0.25 | 37 | 7 | 20 | 35 | 558 |

* 100% Decomposition to picric acid and other products.

Table A-70

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 38

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 11 | 13 | 24 | 121 |
| | + | | 12 | 19 | 29 | 128 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 µg | | | | |
| 9-Aminoacridine | - | 100 | 1165 | | | |
| 2-Nitrofluorene | - | 50 | | 882 | | |
| AF2 | - | 0.1 | | | 300 | 840 |
| 2-Anthramine | + | 20 | 99 | 1166 | 2685 | 2910 |
| Pre-chlorination | - | 0.05 ml | 10 | 15 | 34 | 152 |
| 2,4,6-Trinitrobenzonitrile | - | 0.1 | 16 | 14 | 30 | 137 |
| | - | 0.15 | 7 | 43 | 0 | 222 |
| | - | 0.2 | 12 | 41 | 0 | 218 |
| | - | 0.25 | 11 | 25 | 11 | 230 |
| | + | 0.05 | 8 | 34 | 43 | 98 |
| | + | 0.1 | 13 | 22 | 42 | 102 |
| | + | 0.15 | 13 | 28 | 18 | 75 |
| | + | 0.2 | 19 | 33 | 40 | 116 |
| | + | 0.25 | 14 | 40 | 67 | 176 |

* 100% Decomposition to picric acid and other compounds.

Table A-70 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Post-chlorination 2,4,6-Trinitrobenzonitrile | - | 0.05 ml | 20 | 14 | 50 | 161 |
| | - | 0.1 | 25 | 28 | 81 | 176 |
| | - | 0.15 | 40 | 49 | 130 | 207 |
| | - | 0.20 | 35 | 52 | 163 | 265 |
| | - | 0.25 | 13 | 21 | 130 | 313 |
| | + | 0.05 | 21 | 30 | 36 | 120 |
| | + | 0.1 | 12 | 26 | 48 | 98 |
| | + | 0.15 | 15 | 30 | 33 | 150 |
| | + | 0.2 | 14 | 36 | 50 | 136 |
| | + | 0.25 | 20 | 33 | 88 | 140 |

Table A-71

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 50 | 20 | 41 | 28 | 144 |
| | + | | 19 | 35 | 43 | 54 | 137 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 1500 | | | | |
| 9-Aminoacridine | - | 100 | | 440 | | | |
| 2-Nitrofluorene | - | 50 | | | 324 | | |
| AF2 | - | 0.1 | | | | 600 | 1500 |
| 2-Anthramine | + | 20 | 217 | 500 | 3000 | 1000 | 6000 |
| Pre-ozonation | - | 0.25 ml | 45 | 13 | 22 | 116 | 261 |
| 2,4,6-Trinitrobenzonitrile | + | 0.25 | 32 | 27 | 37 | 83 | 296 |
| Post-ozonation | - | 0.005 ml | 50 | 19 | 40 | 43 | 122 |
| 2,4,6-Trinitrobenzonitrile | - | 0.01 | 32 | 22 | 25 | 36 | 147 |
| | - | 0.025 | 54 | 16 | 22 | 57 | 134 |
| | - | 0.05 | 69 | 18 | 18 | 60 | 204 |
| | - | 0.1 | 45 | 20 | 32 | 80 | 262 |
| | - | 0.25 | 50 | 17 | 31 | 226 | 540 |
| | + | 0.005 | 25 | 28 | 42 | 42 | 146 |
| | + | 0.01 | 22 | 27 | 40 | 58 | 160 |
| | + | 0.025 | 23 | 23 | 40 | 35 | 164 |
| | + | 0.05 | 25 | 28 | 35 | 48 | 165 |
| | + | 0.1 | 26 | 22 | 32 | 77 | 340 |
| | + | 0.25 | 27 | 31 | 41 | 119 | 550 |

* 100% Decomposition to picric acid and other compounds.

Table A-72

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 36

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 28 | 5 | 9 | 22 | 76 |
| | + | | 14 | 2 | 15 | 21 | 82 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 µg | 224 | | | | |
| 9-Aminoacridine | - | 100 | | 900 | | | |
| 2-Nitrofluorene | - | 50 | | | 1300 | | |
| AF2 | - | 0.1 | | | | 451 | 1200 |
| 2-Anthramine | + | 20 | 416 | 322 | 1955 | 2200 | 900 |
| Pre-ozonation | - | 0.005 ml | 23 | 6 | 11 | 20 | C† |
| 2,4,6-Trinitrobenzonitrile | - | 0.01 | 21 | 1 | 12 | 27 | C |
| | - | 0.025 | 18 | 6 | 7 | 27 | C |
| | - | 0.05 | 20 | 2 | 19 | 30 | C |
| | - | 0.1 | 20 | 8 | 20 | 54 | C |
| | - | 0.25 | 22 | 23 | 28 | 72 | C |
| | + | 0.005 | 8 | 4 | 4 | 23 | C |
| | + | 0.01 | 16 | 7 | 9 | 23 | C |
| | + | 0.025 | 32 | 5 | 6 | 18 | C |
| | + | 0.05 | 11 | 5 | 15 | 30 | C |
| | + | 0.1 | 13 | 6 | 12 | 26 | C |
| | + | 0.25 | 4 | 9 | 15 | 70 | C |

* 100% Decomposition to picric acid and other compounds.

† C, Contaminated.

Table A-72 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Post-ozonation* | - | 0.005 ml | 18 | 4 | 6 | 18 | C |
| 2,4,6-Trinitrobenzonitrile | - | 0.01 | 15 | 13 | 8 | 19 | C |
| | - | 0.025 | 11 | 2 | 7 | 15 | C |
| | - | 0.05 | 13 | 7 | 5 | 42 | C |
| | - | 0.1 | 15 | 8 | 15 | 46 | C |
| | - | 0.25 | 11 | 10 | 21 | 110 | C |
| | + | 0.005 | 8 | 0 | 6 | 8 | C |
| | + | 0.01 | 10 | 1 | 6 | 4 | C |
| | + | 0.025 | 13 | 2 | 8 | 14 | C |
| | + | 0.05 | 14 | 3 | 2 | 6 | C |
| | + | 0.1 | 8 | 2 | 6 | 5 | C |
| | + | 0.25 | 7 | 8 | 4 | 9 | C |

* 100% Hydrolysis.

Table A-73

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 40

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Negative control | - | | 115 |
| | + | | 119 |
| Positive controls | | | |
| AF2 | - | 0.1 µg | 446 |
| 2-Anthramine | + | 10 | 1755 |
| Pre-ozonation | - | 0.005 ml | 118 |
| 2,4,6-Trinitrobenzonitrile | - | 0.01 | 117 |
| | - | 0.025 | 137 |
| | - | 0.05 | 141 |
| | - | 0.1 | 152 |
| | - | 0.25 | 160 |
| | + | 0.005 | 124 |
| | + | 0.01 | 139 |
| | + | 0.025 | 106 |
| | + | 0.05 | 132 |
| | + | 0.1 | 159 |
| | + | 0.25 | 153 |

* 100% Decomposition to picric acid and other compounds.

Table A-73 (concluded)

| Compound | Metabolic Activation | Amount of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Post-ozonation | - | 0.005 ml | 133 |
| 2,4,6-Trinitrobenzonitrile | - | 0.01 | 140 |
| | - | 0.025 | 127 |
| | - | 0.05 | 141 |
| | - | 0.1 | 129 |
| | - | 0.25 | 142 |
| | + | 0.005 | 92 |
| | + | 0.01 | 133 |
| | + | 0.025 | 150 |
| | + | 0.05 | 119 |
| | + | 0.1 | 92 |
| | + | 0.25 | 126 |

Table A-74

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 28 | 23 | 29 | 83 |
| | + | | 12 | 40 | 21 | 44 | 108 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 550 | | | | |
| 9-Aminoacridine | - | 100 | | 430 | | | |
| 2-Nitrofluorene | - | 50 | | | 65 | | |
| AF2 | - | 0.1 | | | | 257 | 324 |
| 2-Anthramine | + | 20 | 332 | 850 | 1800 | 1200 | 1900 |
| Pre-chlorination | - | 2.9 | 57 | 36 | 19 | 48 | 99 |
| 2,4,6-Trinitrobenzaldehyde | + | 2.9 | 13 | 40 | 31 | 40 | 136 |
| Post-chlorination | - | 0.06 | 44 | 28 | 22 | 30 | 85 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.12 | 25 | 30 | 19 | 28 | 78 |
| | - | 0.3 | 37 | 25 | 18 | 33 | 74 |
| | - | 0.6 | 40 | 25 | 21 | 28 | 96 |
| | - | 1.2 | 61 | 29 | 28 | 33 | C* |
| | - | 2.9 | 53 | 32 | 27 | 45 | 112 |
| | + | 0.06 | 14 | C | 23 | 40 | 105 |
| | + | 0.12 | 17 | C | 23 | 34 | C |
| | + | 0.3 | 18 | 46 | 29 | 33 | 106 |
| | + | 0.6 | 20 | 46 | 25 | 47 | 121 |
| | + | 1.2 | 26 | C | 33 | 38 | 132 |
| | + | 2.9 | 43 | 36 | 33 | 47 | 120 |

* C, Contaminated.

Table A-75

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 31

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 17 | 11 | 14 | 14 | 164 |
| | + | | 8 | 9 | 15 | 20 | 147 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 650 | | | | |
| 9-Aminoacridine | - | 100 | | 250 | | | |
| 2-Nitrofluorene | - | 50 | | | 291 | | |
| AF2 | - | 0.1 | | | | 21 | 560 |
| 2-Anthramine | + | 20 | 10 | 430 | 1600 | 94 | 1500 |
| Pre-chlorination | - | 15 | 18 | 14 | 51 | 120 | 267 |
| 2,4,6-Trinitrobenzaldehyde | + | 15 | 12 | 8 | 20 | 23 | 230 |
| Post-chlorination | - | 2.2 | 84 | 60 | 60 | 63 | 257 |
| 2,4,6-Trinitrobenzaldehyde | - | 4.4 | 105 | 110 | 128 | 93 | 312 |
| | - | 6.6 | 122 | 189 | 190 | 138 | 379 |
| | - | 8.8 | 119 | 216 | 237 | 180 | 460 |
| | - | 11 | 152 | 227 | 210 | 290 | 455 |
| | + | 2.2 | 91 | 48 | 61 | 61 | 202 |
| | + | 4.4 | 153 | 72 | 120 | 120 | 320 |
| | + | 6.6 | 157 | 133 | 182 | 182 | 360 |
| | + | 8.8 | 209 | 135 | 245 | 230 | 380 |
| | + | 11 | 198 | 219 | 240 | 260 | 380 |

Table A-76

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 37

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Negative control | - | | 7 | 10 | 14 | 92 |
| | + | | 5 | 7 | 18 | 77 |
| Positive controls | | | | | | |
| β-Propiolactone | - | 50 | 262 | | | |
| 9-Aminoacridine | - | 100 | | 1405 | | |
| 2-Nitrofluorene | - | 50 | | | | |
| AF2 | - | 0.1 | | | 400 | 1176 |
| 2-Anthramine | + | 20 | 330 | 247 | 1836 | 1290 |
| Pre-chlorination | - | 57 | 16 | 3 | 3 | 244 |
| 2,4,6-Trinitrobenzaldehyde | + | 57 | 11 | 9 | 86 | 209 |
| Post-chlorination | - | 10 | 11 | 12 | 150 | 274 |
| 2,4,6-Trinitrobenzaldehyde | - | 20 | 22 | 10 | C | 368 |
| | - | 30 | 21 | 3 | 13 | 414 |
| | - | 40 | 20 | 4 | 4 | 247 |
| | - | 50 | 23 | 0 | 9 | 280 |
| | + | 10 | 11 | 6 | 44 | 127 |
| | + | 20 | 10 | 8 | 78 | 177 |
| | + | 30 | 9 | 5 | 92 | 194 |
| | + | 40 | 14 | 8 | 84 | 215 |
| | + | 50 | 13 | 12 | 126 | 221 |

Table A-77

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 45

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 |
|---|---|---|---|
| Negative control | - | | 30 |
| | + | | 37 |
| Positive controls | | | |
| Sodium azide | - | 1.0 | 632 |
| 2-Anthramine | - | 2.5 | 48 |
| | + | 2.5 | 247 |
| Pre-chlorination | - | 14.8 | 75 |
| 2,4,6-Trinitrobenzaldehyde | + | 14.8 | 26 |
| Post-chlorination | - | 2.5 | 35 |
| 2,4,6-Trinitrobenzaldehyde | - | 5.0 | 39 |
| | - | 7.6 | 50 |
| | - | 10.1 | 70 |
| | - | 12.6 | 72 |
| | + | 2.5 | 29 |
| | + | 5.0 | 32 |
| | + | 7.6 | 29 |
| | + | 10.1 | 30 |
| | + | 12.6 | 39 |

Table A-78

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 37 | 28 | 23 | 29 | 83 |
| | + | | 12 | 40 | 21 | 44 | 108 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 550 | | | | |
| 9-Aminoacridine | - | 100 | | 430 | | | |
| 2-Nitrofluorene | - | 50 | | | 65 | | |
| AF2 | - | 0.1 | | | | 257 | 324 |
| 2-Anthramine | + | 20 | 332 | 850 | 1800 | 1200 | 1900 |
| Pre-ozonation | - | 3.2 | 31 | 25 | 14 | 41 | 111 |
| 2,4,6-Trinitrobenzaldehyde | + | 3.2 | 13 | 30 | 33 | 45 | 133 |
| Post-ozonation | - | 0.04 | 17 | 52 | 20 | 26 | 117 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.08 | 15 | 18 | 23 | 30 | 109 |
| | - | 0.2 | 40 | 21 | 22 | 33 | 93 |
| | - | 0.4 | 31 | 31 | 17 | 52 | 115 |
| | - | 0.8 | 30 | 35 | 26 | 42 | 112 |
| | - | 2 | 37 | 21 | 14 | 49 | 122 |
| | + | 0.04 | 15 | 44 | 26 | 37 | 99 |
| | + | 0.08 | 17 | 43 | 27 | 41 | 113 |
| | + | 0.2 | 15 | 39 | 29 | 44 | 120 |
| | + | 0.4 | 21 | 38 | 24 | 38 | 116 |
| | + | 0.8 | 18 | 32 | 36 | 37 | 132 |
| | + | 2 | 16 | 23 | 24 | C* | 146 |

* C, Contaminated.

Table A-79

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 36

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 28 | 5 | 8 | 22 | C* |
| | + | | 14 | 2 | 15 | 21 | C |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 224 | | | | C |
| 9-Aminoacridine | - | 100 | | 900 | | | |
| 2-Nitrofluorene | - | 50 | | | 1300 | | C |
| AF2 | - | 0.1 | | | | 451 | C |
| 2-Anthramine | + | 20 | 416 | 322 | 1955 | 2200 | C |
| Pre-ozonation | - | 17.8 | 43 | 50 | 145 | 246 | C |
| 2,4,6-Trinitrobenzaldehyde | + | 17.8 | 15 | 7 | 15 | 58 | C |
| Post-ozonation | - | 3.2 | 33 | 8 | 31 | 44 | C |
| 2,4,6-Trinitrobenzaldehyde | - | 6.4 | 28 | 22 | 60 | 122 | C |
| | - | 9.6 | 28 | 25 | 77 | 188 | C |
| | - | 12.8 | 33 | 24 | 126 | 290 | C |
| | - | 16 | 35 | 44 | 146 | 300 | C |
| | + | 3.2 | 12 | 6 | 10 | 29 | C |
| | + | 6.4 | 13 | 6 | 12 | 28 | C |
| | + | 9.6 | 15 | 5 | 18 | 40 | C |
| | + | 12.8 | 11 | 6 | 12 | 47 | C |
| | + | 16 | 11 | 7 | 20 | 50 | C |

* C, Contaminated.

Table A-80

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 40

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative control | - | | 20 | 15 | 9 | 19 | 115 |
| | + | | 9 | 14 | 23 | 28 | 119 |
| Positive controls | | | | | | | |
| β-Propiolactone | - | 50 | 456 | | | | |
| 9-Aminoacridine | - | 100 | | 546 | | | |
| 2-Nitrofluorene | - | 50 | | | 1295 | | |
| AF2 | - | 0.1 | | | | 98 | 446 |
| 2-Anthramine | + | 10 | 604 | 444 | 2560 | 2320 | 1755 |
| Pre-ozonation 2,4,6-Trinitrobenzaldehyde | - | 3.3 | 27 | 13 | 48 | 38 | 196 |
| | - | 6.5 | 16 | 15 | 56 | 84 | 249 |
| | - | 9.8 | 24 | 11 | 84 | 150 | 308 |
| | - | 13.1 | 17 | 33 | 137 | 243 | 297 |
| | - | 16.4 | 19 | 22 | 287 | 345 | 407 |
| | + | 3.3 | 12 | 7 | 29 | 17 | 100 |
| | + | 6.5 | 11 | 13 | 25 | 27 | 157 |
| | + | 9.8 | 8 | 15 | 27 | 44 | 178 |
| | + | 13.1 | 20 | 13 | 17 | 38 | 212 |
| | + | 16.4 | 19 | 15 | 24 | 34 | 189 |

Table A-80 (concluded)

| Compound | Metabolic Activation | Micrograms of Compound Added per Plate | Histidine Revertants per Plate TA100 |
|---|---|---|---|
| Post-ozonation | - | 2.7 | 260 |
| 2,4,6-Trinitrobenzaldehyde | - | 5.4 | 350 |
| | - | 8.1 | 390 |
| | - | 10.8 | 463 |
| | - | 13.5 | 452 |
| | + | 2.7 | 167 |
| | + | 5.4 | 192 |
| | + | 8.1 | 244 |
| | + | 10.8 | 298 |
| | + | 13.5 | 293 |

# APPENDIX B

## IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

APPENDIX B--*IN VITRO* ASSAYS WITH *SACCHAROMYCES CEREVISIAE*

Table B-1

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 25

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 6.0 | 10 |
| | + | | 5.0 | 100 | 3.5 | 7.0 |
| Pre-chlorination 1,3,5-Trinitrobenzene | - | 0.007 | 6.2 | 107 | 4.0 | 6.5 |
| | + | 0.007 | 6.3 | 126 | 5.0 | 7.9 |
| Post-chlorination 1,3,5-Trinitrobenzene | - | 0.007 | 6.7 | 116 | 7.0 | 10 |
| | - | 0.0035 | 6.4 | 110 | 5.0 | 7.8 |
| | - | 0.0017 | 6.0 | 103 | 4.0 | 6.7 |
| | - | 0.0007 | 5.9 | 102 | 9.0 | 15 |
| | - | 0.00014 | 6.1 | 105 | 3.0 | 4.9 |
| | + | 0.007 | 6.3 | 126 | 6.0 | 9.5 |
| | + | 0.0035 | 6.1 | 122 | 10.0 | 16 |
| | + | 0.0017 | 5.5 | 110 | 7.0 | 13.0 |
| | + | 0.0007 | 6.3 | 126 | 7.0 | 11.0 |
| | + | 0.00014 | 6.9 | 138 | 6.0 | 8.7 |

The positive control values for the Saccharomyces cerevisiae D3 experiments are presented on Table 148.

Table B-2

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 30

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.1 | 100 | 6.3 | 10 |
| | + | | 6.9 | 100 | 6.3 | 9.1 |
| Pre-chlorination 1,3,5-Trinitrobenzene | - | 0.012 | 7.2 | 118 | 5.0 | 7.0 |
| | + | 0.012 | 7.5 | 109 | 7.0 | 9.3 |
| Post-chlorination 1,3,5-Trinitrobenzene | - | 0.012 | 7.3 | 120 | 6.0 | 8.2 |
| | - | 0.006 | 8.3 | 136 | 3.0 | 3.6 |
| | - | 0.003 | 7.7 | 126 | 15 | 19 |
| | - | 0.0012 | 6.6 | 108 | 5.0 | 7.6 |
| | - | 0.00024 | 6.9 | 113 | 18 | 26 |
| | + | 0.012 | 7.4 | 107 | 8.0 | 11 |
| | + | 0.006 | 8.1 | 117 | 11 | 14 |
| | + | 0.003 | 7.6 | 110 | 6.0 | 7.9 |
| | + | 0.0012 | 9.3 | 135 | 19 | 20 |
| | + | 0.00024 | 8.4 | 122 | 7.0 | 8.3 |

Table B-3

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 26

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml (x $10^{-7}$) | Percent | Per ml (x $10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 12.0 | 100 | 7.5 | 6.3 |
| | + | | 9.1 | 100 | 8.5 | 9.3 |
| Pre-ozonation | - | 0.015 | 4.6 | 38 | 5.0 | 11 |
| 1,3,5-Trinitrobenzene | + | 0.015 | 4.5 | 49 | 5.0 | 11 |
| Post-ozonation | - | 0.014 | 4.6 | 38 | 4.0 | 8.7 |
| 1,3,5-Trinitrobenzene | - | 0.007 | 6.0 | 50 | 5.0 | 8.3 |
| | - | 0.0035 | 5.0 | 42 | 5.0 | 10 |
| | - | 0.0014 | 5.1 | 43 | 6.0 | 12 |
| | - | 0.0003 | 4.0 | 33 | 1.0 | 2.5 |
| | + | 0.014 | 4.7 | 52 | 3.0 | 6.4 |
| | + | 0.007 | 4.5 | 49 | 6.0 | 13 |
| | + | 0.0035 | 4.2 | 46 | 5.0 | 12 |
| | + | 0.0014 | 4.8 | 53 | 5.0 | 10 |
| | + | 0.0003 | 4.0 | 44 | 4.0 | 10 |

Table B-4

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3,5-TRINITROBENZENE

EXPERIMENT 35

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.6 | 100 | 3.8 | 5.8 |
| | + | | 8.0 | 100 | 3.7 | 4.6 |
| Pre-ozonation 1,3,5-Trinitrobenzene | - | 0.014 | 7.1 | 108 | 8.0 | 11 |
| | + | 0.014 | 6.8 | 85 | 5.0 | 7.4 |
| Post-ozonation 1,3,5-Trinitrobenzene | - | 0.014 | 6.7 | 102 | 5.0 | 7.5 |
| | - | 0.0105 | 7.1 | 108 | 4.0 | 5.6 |
| | - | 0.007 | 7.1 | 108 | 8.0 | 11 |
| | - | 0.0035 | 6.3 | 95 | 5.0 | 7.9 |
| | - | 0.0014 | 8.1 | 123 | 4.0 | 4.9 |
| | + | 0.014 | 6.3 | 79 | 3.0 | 4.8 |
| | + | 0.0105 | 8.3 | 104 | 6.3 | 7.6 |
| | + | 0.007 | 8.8 | 110 | 9.0 | 10 |
| | + | 0.0035 | 6.6 | 83 | 9.0 | 14 |
| | + | 0.0014 | C* | C | C | C |

*C, Contaminated

Table B-5

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE

EXPERIMENT 23

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.7 | 100 | 7.0 | 10 |
| | + | | 6.1 | 100 | 10 | 16 |
| Pre-chlorination 1,3-Dinitrobenzene | - | 0.005 | 6.4 | 96 | 6.0 | 9.4 |
| | + | 0.005 | 8.0 | 131 | 5.0 | 6.0 |
| Post-chlorination 1,3-Dinitrobenzene | - | 0.005 | 6.2 | 93 | 6.0 | 9.7 |
| | - | 0.0025 | 6.2 | 93 | 9.0 | 15 |
| | - | 0.0012 | 5.8 | 87 | 6.0 | 10 |
| | - | 0.0005 | 6.1 | 91 | 11 | 18 |
| | - | 0.0001 | 6.9 | 103 | 4.0 | 5.8 |
| | + | 0.005 | 7.8 | 128 | 8.0 | 10 |
| | + | 0.0025 | 5.1 | 84 | 8.0 | 16 |
| | + | 0.0012 | 5.9 | 97 | 6.0 | 10 |
| | + | 0.0005 | 5.0 | 82 | 8.0 | 16 |
| | + | 0.0001 | 6.5 | 107 | 11 | 17 |

Table B-6

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE

EXPERIMENT 30

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.1 | 100 | 6.3 | 10 |
| | + | | 6.9 | 100 | 6.3 | 9.1 |
| Pre-chlorination 1,3-Dinitrobenzene | - | 0.0074 | 7.9 | 130 | 7.0 | 8.9 |
| | + | 0.0074 | 7.8 | 113 | 18 | 23 |
| Post-chlorination 1,3-Dinitrobenzene | - | 0.0074 | C* | C | C | C |
| | - | 0.0037 | 8.2 | 134 | 5.0 | 6.1 |
| | - | 0.0018 | 9.1 | 149 | 5.0 | 5.5 |
| | - | 0.00074 | 6.0 | 98 | 7.0 | 12 |
| | - | 0.00015 | 6.9 | 113 | 14 | 20 |
| | + | 0.0074 | 8.2 | 119 | 11 | 13 |
| | + | 0.0037 | 9.1 | 132 | 6.3 | 6.9 |
| | + | 0.0018 | 8.7 | 126 | 8.0 | 9.2 |
| | + | 0.00074 | 6.4 | 93 | 4.0 | 6.3 |
| | + | 0.00015 | 5.9 | 86 | 6.0 | 10 |

*C, Contaminated

Table B-7

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE

EXPERIMENT 27

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.4 | 100 | 3.5 | 8.0 |
| | + | | 4.6 | 100 | 3.5 | 7.6 |
| Pre-ozonation | - | 0.002 | 10.0 | 227 | 5.0 | 5.0 |
| 1,3-Dinitrobenzene | + | 0.002 | 4.6 | 100 | 3.0 | 6.5 |
| Post-ozonation | - | 0.0015 | 4.2 | 95 | 5.0 | 12 |
| 1,3-Dinitrobenzene | - | 0.0008 | 4.3 | 98 | 2.0 | 4.7 |
| | - | 0.0004 | 5.0 | 114 | 2.0 | 4.0 |
| | - | 0.00015 | 4.4 | 100 | 3.0 | 6.8 |
| | - | 0.00003 | 3.9 | 89 | 1.3 | 3.3 |
| | + | 0.0015 | 4.4 | 96 | 1.0 | 2.3 |
| | + | 0.0008 | 5.4 | 117 | 9.0 | 17 |
| | + | 0.0004 | 6.4 | 139 | 3.0 | 4.7 |
| | + | 0.00015 | 4.5 | 98 | 4.0 | 8.9 |
| | + | 0.00003 | 4.1 | 89 | 6.0 | 15 |

Table B-8

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
1,3-DINITROBENZENE

EXPERIMENT 36

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 7.6 | 100 | 5.0 | 6.6 |
| | + | | 8.8 | 100 | 7.0 | 8.0 |
| Pre-ozonation 1,3-Dinitrobenzene | - | 0.004 | 7.9 | 104 | 5.0 | 6.3 |
| | + | 0.004 | 6.8 | 77 | 8.8 | 13 |
| Post-ozonation 1,3-Dinitrobenzene | - | 0.0036 | 7.4 | 97 | 3.0 | 4.1 |
| | - | 0.0027 | 7.2 | 95 | 8.0 | 11 |
| | - | 0.0018 | 7.9 | 104 | 5.0 | 6.3 |
| | - | 0.0009 | 6.4 | 84 | 5.0 | 7.8 |
| | - | 0.00036 | 5.2 | 68 | 6.3 | 12 |
| | + | 0.0036 | 7.0 | 80 | 5.0 | 7.1 |
| | + | 0.0027 | 7.3 | 83 | 3.0 | 4.1 |
| | + | 0.0018 | 7.9 | 90 | 6.7 | 8.5 |
| | + | 0.0009 | 7.5 | 85 | 11 | 15 |
| | + | 0.00036 | 6.2 | 70 | 3.8 | 6.1 |

Table B-9

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
TRINITROGLYCERINE*

EXPERIMENT 25

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.8 | 100 | 6.0 | 10 |
| | + | | 5.0 | 100 | 3.5 | 7.0 |
| Pre-chlorination Trinitroglycerine | - | 1 | 6.2 | 107 | 2.0 | 3.2 |
| | + | 1 | 6.3 | 126 | 4.0 | 6.3 |
| Post-chlorination Trinitroglycerine | - | 1 | 6.5 | 112 | 6.0 | 9.2 |
| | - | 0.5 | 6.6 | 114 | 7.0 | 11.0 |
| | - | 0.25 | 6.3 | 109 | 6.0 | 9.5 |
| | - | 0.1 | 6.7 | 116 | 14 | 21 |
| | - | 0.02 | 5.5 | 95 | 3.0 | 5.5 |
| | + | 1 | 6.2 | 124 | 3.0 | 4.8 |
| | + | 0.5 | 6.4 | 128 | 1.0 | 1.6 |
| | + | 0.25 | 6.4 | 128 | 2.0 | 3.1 |
| | + | 0.1 | 5.9 | 118 | 1.0 | 1.7 |
| | + | 0.02 | 5.8 | 116 | 7.0 | 12 |

*Saturated solution

Table B-10

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
TRINITROGLYCERINE*

EXPERIMENT 34

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 13 | 22 |
| | + | | 5.2 | 100 | 5.8 | 11 |
| Pre-chlorination Trinitroglycerine | - | 1 | 5.7 | 98 | 11 | 19 |
| | + | 1 | 5.3 | 98 | 13 | 25 |
| Post-chlorination Trinitroglycerine | - | 1 | 5.3 | 91 | 12 | 23 |
| | - | 0.75 | 7.8 | 134 | 16 | 21 |
| | - | 0.50 | † | † | † | † |
| | - | 0.25 | 6.4 | 110 | 7.5 | 12 |
| | - | 0.1 | 6.0 | 103 | 13 | 22 |
| | + | 1 | 4.5 | 87 | 8.0 | 18 |
| | + | 0.75 | 4.7 | 90 | 7.0 | 15 |
| | + | 0.50 | 5.2 | 100 | 8.0 | 15 |
| | + | 0.25 | 6.3 | 121 | 3.0 | 4.8 |
| | + | 0.1 | 9.3 | 179 | 9.0 | 9.7 |

*Saturated solution
†Dilution error

Table B-11

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.9 | 100 | 5.5 | 9.3 |
| | + | | 5.4 | 100 | 6.5 | 12 |
| Pre-chlorination PETN | - | 1 | 6.1 | 103 | 6.0 | 9.8 |
| | + | 1 | 6.0 | 111 | 2.0 | 3.3 |
| Post-chlorination PETN | - | 1 | 4.9 | 83 | 4.0 | 8.2 |
| | - | 0.5 | 5.2 | 88 | 4.0 | 7.7 |
| | - | 0.25 | 4.6 | 78 | 7.0 | 15 |
| | - | 0.1 | 6.1 | 103 | 4.0 | 6.6 |
| | - | 0.02 | 6.1 | 103 | 7.0 | 11 |
| | + | 1 | 9.2 | 170 | 6.0 | 6.5 |
| | + | 0.5 | 5.2 | 96 | 7.0 | 13 |
| | + | 0.25 | 5.5 | 102 | 5.0 | 9.1 |
| | + | 0.1 | 6.4 | 119 | 7.0 | 11 |
| | + | 0.02 | 6.1 | 113 | 5.0 | 8.2 |

*Saturated solution

Table B-12

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 32

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.0 | 100 | 5.5 | 11 |
| | + | | 5.7 | 100 | 2.5 | 4.4 |
| Pre-chlorination PETN | - | 1 | 5.6 | 112 | 4.0 | 7.1 |
| | + | 1 | 4.8 | 84 | 4.0 | 8.3 |
| Post-chlorination PETN | - | 1 | 5.4 | 108 | 3.0 | 5.6 |
| | - | 0.75 | 5.7 | 114 | 11 | 19 |
| | - | 0.5 | 5.5 | 110 | 2.0 | 3.6 |
| | - | 0.25 | 6.4 | 128 | 2.0 | 3.1 |
| | - | 0.1 | 5.8 | 116 | 3.0 | 5.2 |
| | + | 1 | 5.7 | 100 | 5.0 | 8.8 |
| | + | 0.75 | 6.4 | 112 | 1.0 | 1.6 |
| | + | 0.5 | 5.5 | 96 | 4.0 | 7.3 |
| | + | 0.25 | 6.4 | 112 | 2.0 | 3.1 |
| | + | 0.1 | 5.2 | 91 | 2.0 | 3.8 |

*Saturated solution

Table B-13

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 26

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 12.0 | 100 | 7.5 | 6.3 |
| | + | | 9.1 | 100 | 8.5 | 9.3 |
| Pre-ozonation PETN | - | 1 | 5.2 | 43 | 2.0 | 3.8 |
| | + | 1 | 4.8 | 53 | 11 | 23 |
| Post-ozonation PETN | - | 1 | 8.0 | 67 | 4.0 | 5.0 |
| | - | 0.5 | 5.3 | 44 | 7.0 | 13 |
| | - | 0.25 | 5.6 | 47 | 11 | 20 |
| | - | 0.1 | 5.2 | 43 | 9.0 | 17 |
| | - | 0.02 | 4.9 | 41 | 5.0 | 10 |
| | + | 1 | 5.5 | 60 | 8.0 | 15 |
| | + | 0.5 | 5.1 | 56 | 3.0 | 5.9 |
| | + | 0.25 | 7.2 | 79 | 7.0 | 9.7 |
| | + | 0.1 | 4.8 | 53 | 8.0 | 17 |
| | + | 0.02 | 4.8 | 53 | 4.0 | 8.3 |

*Saturated solution

Table B-14

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
PENTAERYTHRITOL TETRANITRATE*

EXPERIMENT 35

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 6.6 | 100 | 3.8 | 5.8 |
| | + | | 8.0 | 100 | 3.7 | 4.6 |
| Pre-ozonation PETN | - | 1 | 6.8 | 103 | 3.0 | 4.4 |
| | + | 1 | 7.5 | 94 | 3.0 | 4.0 |
| Post-ozonation PETN | - | 1 | 7.2 | 109 | 4.0 | 5.6 |
| | - | 0.75 | 6.2 | 94 | 6.0 | 9.7 |
| | - | 0.5 | 7.2 | 109 | 5.0 | 6.9 |
| | - | 0.25 | 6.8 | 103 | 5.0 | 7.4 |
| | - | 0.1 | 6.9 | 105 | 7.0 | 10 |
| | + | 1 | 6.9 | 86 | 6.0 | 8.7 |
| | + | 0.75 | 5.0 | 63 | 7.0 | 14 |
| | + | 0.5 | 7.5 | 94 | 5.0 | 6.7 |
| | + | 0.25 | 5.5 | 69 | 5.0 | 9.1 |
| | + | 0.1 | 8.5 | 106 | 5.0 | 5.9 |

*Saturated solution

Table B-15

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
CONDENSATE WATER

EXPERIMENT 19

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 7.8 | 100 | 3.0 | 3.9 |
| | + | | 6.6 | 100 | 3.0 | 4.6 |
| Pre-chlorination | - | 0.0037 | 7.3 | 94 | 7.0 | 9.6 |
| Condensate water | + | 0.0037 | 6.4 | 98 | 6.0 | 9.4 |
| Post-chlorination | - | 0.0037 | 6.5 | 84 | 6.0 | 9.2 |
| Condensate water | - | 0.0019 | 6.9 | 88 | 3.0 | 4.4 |
| | - | 0.0009 | 7.1 | 91 | 1.0 | 1.4 |
| | - | 0.00037 | 6.7 | 86 | 6.0 | 9.0 |
| | - | 0.00007 | 7.5 | 97 | 7.0 | 9.3 |
| | + | 0.0037 | 7.4 | 112 | 4.0 | 5.4 |
| | + | 0.0019 | 7.1 | 108 | 6.0 | 8.5 |
| | + | 0.0009 | 5.9 | 90 | 9.0 | 15 |
| | + | 0.00037 | 7.1 | 108 | 2.0 | 2.8 |
| | + | 0.00007 | 7.0 | 106 | 1.0 | 1.4 |

Table B-16

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
CONDENSATE WATER

EXPERIMENT 29

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.3 | 100 | 5.0 | 9.4 |
| | + | | 5.9 | 100 | 3.5 | 6.0 |
| Pre-chlorination | - | 0.0027 | 5.2 | 98 | 2.0 | 3.9 |
| Condensate water | + | 0.0027 | 5.6 | 95 | 4.0 | 7.1 |
| Post-chlorination | - | 0.0027 | 4.1 | 77 | 3.0 | 7.3 |
| Condensate water | - | 0.002 | 5.6 | 106 | 3.0 | 5.4 |
| | - | 0.0014 | 4.6 | 87 | 8.0 | 17 |
| | - | 0.0007 | 6.0 | 113 | 3.3 | 5.5 |
| | - | 0.00027 | 5.3 | 100 | 5.0 | 9.4 |
| | + | 0.0027 | 5.2 | 88 | 1.0 | 1.9 |
| | + | 0.002 | 4.8 | 81 | 6.0 | 13 |
| | + | 0.0014 | 5.5 | 93 | 5.0 | 9.1 |
| | + | 0.0007 | 6.1 | 103 | 8.0 | 13 |
| | + | 0.00027 | 4.5 | 76 | 7.5 | 17 |

Table B-17

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
SYRINGALDAZINE*

EXPERIMENT 20

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 6.8 | 100 | 6.0 | 8.9 |
| | + | | 6.6 | 100 | 2.5 | 3.8 |
| Pre-chlorination Syringaldazine | - | 1 | 7.3 | 108 | 5.0 | 6.9 |
| | + | 1 | 6.2 | 93 | 1.0 | 1.6 |
| Post-chlorination Syringaldazine | - | 1 | 6.2 | 91 | 4.0 | 6.5 |
| | - | 0.5 | 6.2 | 91 | 4.0 | 6.5 |
| | - | 0.25 | 5.6 | 83 | 3.0 | 5.4 |
| | - | 0.1 | 6.1 | 91 | 8.0 | 13 |
| | - | 0.02 | C† | C | C | C |
| | + | 1 | 7.1 | 107 | 6.0 | 8.5 |
| | + | 0.5 | 6.6 | 99 | 3.0 | 4.6 |
| | + | 0.25 | 4.3 | 66 | 4.0 | 9.2 |
| | + | 0.1 | 6.3 | 96 | 8.3 | 13 |
| | + | 0.02 | 5.5 | 83 | 9.0 | 16 |

*Compound photolyzed.
†C, Contaminated.

Table B-18

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
SYRINGALDAZINE

EXPERIMENT 33

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.6 | 100 | 9.9 | 22 |
| | + | | 4.8 | 100 | 12 | 25 |
| Pre-chlorination Syringaldazine | - | 0.0049 | 4.1 | 89 | 2.0 | 4.9 |
| | + | 0.0049 | 4.4 | 92 | <1.0 | 2.3 |
| Post-chlorination Syringaldazine | - | 0.0032 | 9.1 | 198 | 6.0 | 6.6 |
| | - | 0.0024 | 5.1 | 111 | 7.0 | 14 |
| | - | 0.0016 | 5.2 | 113 | 10 | 19 |
| | - | 0.0008 | C* | C | C | C |
| | - | 0.00032 | 5.9 | 128 | 5.0 | 8.5 |
| | + | 0.0032 | 5.7 | 119 | 8.8 | 15 |
| | + | 0.0024 | 4.8 | 100 | 8.0 | 17 |
| | + | 0.0016 | 4.4 | 92 | 6.0 | 14 |
| | + | 0.0008 | C | C | C | C |
| | + | 0.00032 | 4.4 | 92 | 7.5 | 17 |

*C, Contaminated

Table B-19

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
HMX*
EXPERIMENT 21

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.5 | 100 | 6.5 | 10 |
| | + | | 6.0 | 100 | 5.0 | 8.3 |
| Pre-chlorination HMX | - | 1 | 7.2 | 111 | 7.0 | 9.7 |
| | + | 1 | 7.3 | 120 | 9.0 | 12 |
| Post-chlorination HMX | - | 1 | 6.8 | 105 | 8.0 | 12 |
| | - | 0.5 | 6.5 | 100 | 10 | 15 |
| | - | 0.25 | 7.7 | 118 | 8.0 | 10 |
| | - | 0.1 | 8.4 | 129 | 13 | 15 |
| | - | 0.02 | 9.2 | 142 | 5.0 | 5.4 |
| | + | 1 | 6.6 | 110 | 4.0 | 6.0 |
| | + | 0.5 | 6.5 | 107 | 5.0 | 7.7 |
| | + | 0.25 | 7.4 | 123 | 5.0 | 6.7 |
| | + | 0.1 | 7.3 | 121 | 6.0 | 8.2 |
| | + | 0.02 | 7.2 | 119 | 6.0 | 8.3 |

*Saturated solution

Table B-20

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
HMX*

EXPERIMENT 30

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 6.1 | 100 | 6.3 | 10 |
| | + | | 6.9 | 100 | 6.3 | 9.1 |
| Pre-chlorination HMX | - | 1 | 6.2 | 102 | 6.0 | 9.7 |
| | + | 1 | 6.1 | 88 | 6.0 | 9.9 |
| Post-chlorination HMX | - | 1 | 7.2 | 118 | 4.0 | 5.6 |
| | - | 0.5 | 7.1 | 116 | 3.8 | 5.3 |
| | - | 0.25 | 9.8 | 161 | 4.0 | 4.1 |
| | - | 0.1 | 8.2 | 134 | 6.0 | 7.3 |
| | - | 0.02 | 6.1 | 100 | 5.0 | 8.2 |
| | + | 1 | 6.3 | 91 | 1.0 | 1.6 |
| | + | 0.5 | 6.1 | 88 | 6.0 | 9.9 |
| | + | 0.25 | 7.9 | 114 | 10 | 13 |
| | + | 0.1 | 9.3 | 135 | 2.5 | 2.7 |
| | + | 0.02 | 7.0 | 101 | 2.0 | 2.9 |

*Saturated solution

Table B-21

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE

RDX

EXPERIMENT 21

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.5 | 100 | 6.5 | 10 |
| | + | | 6.0 | 100 | 5.0 | 8.3 |
| Pre-chlorination RDX | - | 0.0019 | 7.2 | 111 | 7.0 | 9.7 |
| | + | 0.0019 | 5.7 | 94 | 3.0 | 5.3 |
| Post-chlorination RDX | - | 0.0019 | 6.0 | 92 | 6.0 | 10 |
| | - | 0.0010 | 6.2 | 95 | 4.0 | 6.5 |
| | - | 0.0005 | 6.5 | 100 | 4.0 | 6.1 |
| | - | 0.00019 | 6.2 | 96 | 11 | 18 |
| | - | 0.00004 | C* | C | C | C |
| | + | 0.0019 | 5.8 | 96 | 6.0 | 10 |
| | + | 0.0010 | 6.0 | 99 | 8.0 | 13 |
| | + | 0.0005 | 6.1 | 101 | 7.0 | 11 |
| | + | 0.0019 | C | C | C | C |
| | + | 0.0004 | C | C | C | C |

*C, Contaminated

Table B-22

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
RDX

EXPERIMENT 29

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.3 | 100 | 5.0 | 9.4 |
| | + | | 5.9 | 100 | 3.5 | 6.0 |
| Pre-chlorination RDX | - | 0.0023 | 6.0 | 113 | 3.0 | 5.0 |
| | + | 0.0023 | 6.6 | 112 | 4.0 | 6.1 |
| Post-chlorination RDX | - | 0.0023 | 7.1 | 134 | 4.0 | 5.7 |
| | - | 0.0017 | 5.0 | 94 | 2.0 | 4.0 |
| | - | 0.0011 | 6.2 | 117 | 5.0 | 8.1 |
| | - | 0.0006 | 5.3 | 100 | 1.0 | 1.9 |
| | - | 0.00023 | 6.5 | 123 | 1.0 | 1.5 |
| | + | 0.0023 | 5.8 | 98 | 4.0 | 6.9 |
| | + | 0.0017 | 5.0 | 85 | 5.0 | 9.9 |
| | + | 0.0011 | 4.9 | 83 | 5.0 | 10 |
| | + | 0.0006 | 6.0 | 102 | 1.0 | 1.7 |
| | + | 0.00023 | 6.4 | 121 | 4.0 | 6.3 |

Table 3-23

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 19

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 7.8 | 100 | 3.0 | 3.9 |
| | + | | 6.6 | 100 | 3.0 | 4.6 |
| Pre-chlorination | - | 1 | 1.6 | 20 | 2.0 | 13 |
| DPO | + | 1 | 3.7 | 56 | 6.0 | 16 |
| Post-chlorination | - | 1 | 1.1 | 14 | 1.0 | 9.1 |
| DPO | - | 0.5 | 6.2 | 80 | 4.0 | 6.4 |
| | - | 0.25 | 5.4 | 69 | 3.0 | 5.6 |
| | - | 0.1 | 6.0 | 77 | 4.0 | 6.7 |
| | - | 0.02 | 5.8 | 74 | 3.0 | 5.2 |
| | + | 1 | 2.4 | 37 | 2.0 | 8.3 |
| | + | 0.5 | 5.4 | 82 | 4.0 | 7.4 |
| | + | 0.25 | 6.0 | 92 | 3.0 | 5.0 |
| | + | 0.1 | 5.8 | 89 | 4.0 | 6.9 |
| | + | 0.02 | 6.0 | 91 | 4.0 | 6.7 |

*Compound photolyzed

Table B-24

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.9 | 100 | 5.5 | 9.3 |
| | + | | 5.3 | 100 | 6.5 | 12 |
| Pre-chlorination | - | 1 | T† | T | T | T |
| DPO | + | 1 | 2.3 | 43 | 2.0 | 8.7 |
| Post-chlorination | - | 1 | T | T | T | T |
| DPO | - | 0.5 | T | T | T | T |
| | - | 0.25 | 0.7 | 12 | 1.0 | 14 |
| | - | 0.1 | 3.8 | 64 | 2.0 | 5.3 |
| | - | 0.02 | 5.2 | 88 | 6.0 | 12 |
| | + | 1 | 1.9 | 36 | 4.0 | 21 |
| | + | 0.5 | 1.6 | 30 | 2.0 | 13 |
| | + | 0.25 | 3.7 | 70 | 6.0 | 16 |
| | + | 0.1 | 7.0 | 132 | 5.0 | 7.1 |
| | + | 0.02 | 4.4 | 83 | 4.0 | 9.1 |

*Compound photolyzed
†T, Toxic

Table B-25

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIETHYL-p-PHENYLENEDIAMINE OXALATE

EXPERIMENT 33

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.6 | 100 | 9.9 | 22 |
| | + | | 4.8 | 100 | 12 | 25 |
| Pre-chlorination | - | 0.009 | 5.1 | 111 | 8.0 | 16 |
| DPO | + | 0.009 | 4.8 | 100 | 6.3 | 13 |
| Post-chlorination | - | 0.0072 | 4.3 | 93 | 6.0 | 14 |
| DPO | - | 0.0054 | 4.9 | 107 | 7.0 | 14 |
| | - | 0.0036 | 4.8 | 104 | 4.0 | 8.3 |
| | - | 0.0018 | 5.1 | 111 | 11 | 22 |
| | - | 0.00072 | 5.0 | 109 | 12 | 24 |
| | + | 0.0072 | 4.4 | 92 | 5.0 | 11 |
| | + | 0.0054 | 5.0 | 104 | 7.0 | 14 |
| | + | 0.0036 | 4.7 | 98 | 9.0 | 19 |
| | + | 0.0018 | 5.1 | 106 | 4.0 | 7.8 |
| | + | 0.00072 | 5.1 | 106 | 4.0 | 7.8 |

Table B-26

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 19

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 7.8 | 100 | 3.0 | 3.9 |
| | + | | 6.6 | 100 | 3.0 | 4.6 |
| Pre-chlorination DPS | - | 1 | 7.5 | 96 | 8.0 | 11 |
| | + | 1 | 5.4 | 82 | 4.0 | 7.5 |
| Post-chlorination DPS | - | 1 | 6.6 | 85 | 3.0 | 4.6 |
| | - | 0.5 | 6.9 | 88 | 1.0 | 1.4 |
| | - | 0.25 | 6.1 | 78 | 4.0 | 6.6 |
| | - | 0.1 | 8.3 | 106 | 1.0 | 1.2 |
| | - | 0.02 | 7.9 | 101 | 1.0 | 1.3 |
| | + | 1 | 5.5 | 83 | 6.0 | 11 |
| | + | 0.5 | 7.6 | 115 | 6.0 | 7.9 |
| | + | 0.25 | 6.1 | 92 | 7.0 | 11 |
| | + | 0.1 | 8.0 | 121 | 2.0 | 2.5 |
| | + | 0.02 | 7.9 | 120 | 2.0 | 2.5 |

*Compound photolyzed

Table B-27

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE*

EXPERIMENT 24

| Compound | Metabolic Activation | Milliliters Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.9 | 100 | 5.5 | 9.3 |
| | + | | 5.3 | 100 | 6.5 | 12 |
| Pre-chlorination | - | 1 | T† | T | T | T |
| DPS | + | 1 | 2.8 | 47 | 6.0 | 21 |
| Post-chlorination | - | 1 | T | T | T | T |
| DPS | - | 0.5 | T | T | T | T |
| | - | 0.25 | 1.2 | 20 | 1.0 | 8.3 |
| | - | 0.1 | 3.7 | 63 | 4.0 | 11 |
| | - | 0.02 | 13 | 22 | 4.0 | 3.1 |
| | + | 1 | 2.4 | 45 | 1.0 | 4.2 |
| | + | 0.5 | 1.3 | 25 | 1.0 | 7.7 |
| | + | 0.25 | 2.5 | 47 | 3.0 | 12 |
| | + | 0.1 | 4.9 | 92 | 4.0 | 8.2 |
| | + | 0.02 | 4.7 | 89 | 2.0 | 4.3 |

*Compound photolyzed
†T, Toxic

Table B-28

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
N,N-DIMETHYL-p-PHENYLENEDIAMINE SULFATE

EXPERIMENT 33

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.6 | 100 | 9.9 | 22 |
| | + | | 4.8 | 100 | 12 | 25 |
| Pre-chlorination | - | 0.0056 | 4.7 | 102 | 15 | 32 |
| DPS | + | 0.0056 | 5.2 | 108 | 11 | 21 |
| Post-chlorination | - | 0.004 | 3.9 | 85 | 13 | 33 |
| DPS | - | 0.003 | 4.3 | 93 | 11 | 26 |
| | - | 0.002 | 5.0 | 109 | 10 | 20 |
| | - | 0.001 | 4.3 | 93 | 9.0 | 21 |
| | - | 0.0004 | 5.0 | 109 | 5.0 | 10 |
| | + | 0.004 | 4.4 | 92 | 8.0 | 18 |
| | + | 0.003 | 4.6 | 96 | 11 | 24 |
| | + | 0.002 | 7.5 | 156 | 7.0 | 9.3 |
| | + | 0.001 | 4.6 | 96 | 6.0 | 13 |
| | + | 0.0004 | 4.8 | 100 | 7.0 | 15 |

Table B-29

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 20

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.8 | 100 | 6.0 | 8.9 |
| | + | | 6.6 | 100 | 2.5 | 3.8 |
| Pre-chlorination 7-50 LAP | - | 0.0068 | 5.6 | 83 | 8.0 | 14 |
| | + | 0.0068 | 4.9 | 75 | 12 | 24 |
| Post-chlorination 7-50 LAP | - | 0.0068 | 5.9 | 88 | 2.0 | 3.4 |
| | - | 0.0034 | 6.8 | 100 | 3.0 | 4.4 |
| | - | 0.0017 | 5.5 | 81 | 7.0 | 13 |
| | - | 0.00068 | 4.7 | 69 | 7.0 | 15 |
| | - | 0.00014 | 5.8 | 86 | 4.0 | 6.9 |
| | + | 0.0068 | 5.6 | 85 | 5.0 | 9.0 |
| | + | 0.0034 | 4.7 | 71 | 6.0 | 13 |
| | + | 0.0017 | 4.9 | 74 | 6.0 | 12 |
| | + | 0.00068 | 5.5 | 83 | 4.0 | 7.3 |
| | + | 0.00014 | 4.8 | 73 | 8.0 | 17 |

Table B-30

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 29

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.3 | 100 | 5.0 | 9.4 |
| | + | | 5.9 | 100 | 3.5 | 6.0 |
| Pre-chlorination 7-50 LAP | - | 0.0014 | 9.9 | 187 | 3.8 | 3.8 |
| | + | 0.0014 | 5.2 | 88 | 4.0 | 7.7 |
| Post-chlorination 7-50 LAP | - | 0.0014 | 5.1 | 96 | 6.3 | 12 |
| | - | 0.0011 | 5.8 | 109 | 3.0 | 5.2 |
| | - | 0.00070 | 5.4 | 102 | 3.0 | 5.6 |
| | - | 0.00035 | 6.0 | 113 | 6.0 | 10 |
| | - | 0.00014 | 5.5 | 104 | 1.0 | 1.8 |
| | + | 0.0014 | 5.1 | 86 | 3.0 | 5.9 |
| | + | 0.0011 | 5.0 | 85 | 2.0 | 4.0 |
| | + | 0.00070 | 5.6 | 95 | 4.0 | 7.2 |
| | + | 0.00035 | 5.6 | 95 | 5.0 | 9.0 |
| | + | 0.00014 | 6.0 | 102 | 2.0 | 3.4 |

Table B-31

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 25

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 6.0 | 10 |
| | + | | 5.0 | 100 | 3.5 | 7.0 |
| Pre-ozonation 7-50 LAP | - | 0.00024 | 4.3 | 74 | 8.0 | 19 |
| | + | 0.00024 | 4.7 | 94 | 5.0 | 11 |
| Post-ozonation 7-50 LAP | - | 0.000023 | 5.8 | 100 | 6.0 | 10 |
| | - | 0.000011 | 5.2 | 90 | 6.0 | 12 |
| | - | 0.000006 | 6.5 | 112 | 2.0 | 3.1 |
| | - | 0.000002 | 5.5 | 95 | 6.0 | 11 |
| | - | 0.0000005 | 4.4 | 76 | 5.0 | 11 |
| | + | 0.000023 | 5.1 | 102 | 6.0 | 12 |
| | + | 0.000011 | 4.9 | 98 | 1.0 | 2.0 |
| | + | 0.000006 | 4.8 | 96 | 1.0 | 2.1 |
| | + | 0.000002 | 4.8 | 96 | 4.0 | 8.3 |
| | + | 0.0000005 | 4.9 | 98 | 8.0 | 16 |

Table B-32

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 34

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 13 | 22 |
| | + | | 5.2 | 100 | 5.8 | 11 |
| Pre-ozonation 7-50 LAP | - | 0.0016 | 6.2 | 107 | 13 | 21 |
| | + | 0.0016 | 5.5 | 106 | 18 | 33 |
| Post-ozonation 7-50 LAP | - | 0.0011 | 5.4 | 93 | 16 | 30 |
| | - | 0.0008 | 5.9 | 102 | 8.0 | 14 |
| | - | 0.0006 | 5.4 | 93 | 11 | 20 |
| | - | 0.0003 | 5.7 | 98 | 7.0 | 12 |
| | - | 0.0001 | 2.9 | 50 | 5.0 | 17 |
| | + | 0.0011 | 6.5 | 125 | 7.0 | 11 |
| | + | 0.0008 | 7.1 | 137 | 11 | 15 |
| | + | 0.0006 | 4.7 | 90 | 3.0 | 6.4 |
| | + | 0.0003 | 6.0 | 115 | 4.0 | 6.7 |
| | + | 0.0001 | 4.6 | 88 | 6.0 | 13 |

Table B-33

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-50 LAP

EXPERIMENT 41

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.7 | 100 | 2.0 | 3.5 |
| | + | | 5.5 | 100 | 7.0 | 13 |
| Pre-ozonation | - | .0013 | 7.0 | 123 | 3.0 | 4.3 |
| 7-50 LAP | + | .0013 | 5.1 | 93 | 4.0 | 7.8 |
| Post-ozonation | - | .0012 | 6.9 | 121 | 7.0 | 10 |
| 7-50 LAP | - | .0006 | 6.1 | 107 | 10 | 16 |
| | - | .0003 | 7.1 | 125 | 7.0 | 9.8 |
| | - | .0001 | 5.6 | 98 | 6.0 | 11 |
| | + | .0012 | 4.7 | 85 | 5.0 | 11 |
| | + | .0006 | 5.1 | 93 | 2.0 | 3.9 |
| | + | .0003 | 5.4 | 98 | 6.0 | 11 |
| | + | .0001 | 6.5 | 118 | 6.0 | 9.2 |

Table B-34

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 20

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 6.8 | 100 | 6.0 | 8.9 |
| | + | | 6.6 | 100 | 2.5 | 3.8 |
| Pre-chlorination | - | 1 | 6.4 | 95 | 12 | 19 |
| 7-100 LAP | + | 1 | 5.8 | 88 | 6.0 | 10 |
| Post-chlorination | - | 1 | 5.0 | 74 | 6.0 | 12 |
| 7-100 LAP | - | 0.5 | 6.2 | 92 | 1.0 | 1.6 |
| | - | 0.25 | 6.8 | 101 | 2.0 | 2.9 |
| | - | 0.1 | 7.3 | 109 | 2.0 | 2.7 |
| | - | 0.02 | 6.7 | 99 | 6.0 | 3.0 |
| | + | 1 | C† | C | C | C |
| | + | 0.5 | 5.6 | 85 | 4.0 | 7.1 |
| | + | 0.25 | 6.8 | 103 | 3.0 | 4.4 |
| | + | 0.1 | 6.5 | 99 | 9.0 | 14 |
| | + | 0.02 | 7.2 | 109 | 4.0 | 5.6 |

*Solution saturated
†C, Contaminated

Table B-35

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 44

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 4.9 | 100 | 4.5 | 9.2 |
| | + | | 4.7 | 100 | 2.0 | 4.3 |
| Pre-chlorination | - | 1.0 | 4.9 | 100 | 6.0 | 12 |
| 7-100 LAP | + | 1.0 | 5.3 | 113 | <1.0 | 1.9 |
| Post-chlorination | - | 1.0 | 4.0 | 82 | 6.0 | 15 |
| 7-100 LAP | - | 0.5 | 5.1 | 104 | 4.0 | 7.9 |
| | - | 0.25 | 5.4 | 110 | 9.0 | 17 |
| | - | 0.1 | 6.6 | 135 | 4.0 | 6.0 |
| | - | 0.02 | 3.2 | 65 | 4.0 | 13 |
| | + | 1.0 | 4.8 | 102 | 7.0 | 15 |
| | + | 0.5 | 3.5 | 74 | 4.0 | 11 |
| | + | 0.25 | † | † | † | † |
| | + | 0.1 | 4.9 | 104 | 2.0 | 4.1 |
| | + | 0.02 | 5.2 | 111 | 4.0 | 7.7 |

*100% Photolyzed
†Dilution error

Table B-36

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 25

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.8 | 100 | 6.0 | 10 |
| | + | | 5.0 | 100 | 3.5 | 7.0 |
| Pre-ozonation 7-100 LAP | - | 1 | 4.8 | 83 | 3.0 | 6.3 |
| | + | 1 | 4.6 | 92 | 10 | 22 |
| Post-ozonation 7-100 LAP | - | 1 | 5.2 | 90 | 5.0 | 9.6 |
| | - | 0.5 | 5.2 | 90 | 6.0 | 12 |
| | - | 0.25 | 5.4 | 93 | 2.0 | 3.7 |
| | - | 0.1 | 5.0 | 86 | 3.0 | 6.0 |
| | - | 0.02 | 5.1 | 88 | 8.0 | 16 |
| | + | 1 | 4.7 | 94 | 4.0 | 8.5 |
| | + | 0.5 | 4.2 | 84 | 4.0 | 9.5 |
| | + | 0.25 | 4.8 | 96 | 5.0 | 10 |
| | + | 0.1 | 5.8 | 116 | 6.0 | 10 |
| | + | 0.02 | 5.2 | 104 | 6.0 | 12 |

*Saturated solution

Table B-37

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
7-100 LAP*

EXPERIMENT 34

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-5}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 5.8 | 100 | 13 | 22 |
| | + | | 5.2 | 100 | 5.8 | 11 |
| Pre-ozonation 7-100 LAP | - | 1 | 6.6 | 114 | 10 | 15 |
| | + | 1 | 5.8 | 112 | 8.0 | 14 |
| Post-ozonation 7-100 LAP | - | 1 | 5.3 | 91 | 8.0 | 15 |
| | - | 0.75 | 4.5 | 78 | 5.0 | 11 |
| | - | 0.5 | 4.1 | 71 | 11 | 27 |
| | - | 0.25 | 5.7 | 98 | 14 | 25 |
| | - | 0.1 | 5.2 | 90 | 15 | 29 |
| | + | 1 | 4.3 | 83 | 9.0 | 21 |
| | + | 0.75 | 4.9 | 94 | 14 | 29 |
| | + | 0.5 | 7.0 | 135 | 10 | 14 |
| | + | 0.25 | C† | C | C | C |
| | + | 0.1 | 4.7 | 90 | 6.7 | 14 |

*Saturated solution
†C, Contaminated

Table B-38

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
9-100 LAP*

EXPERIMENT 44

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 4.9 | 100 | 4.5 | 9.2 |
| | + | | 4.7 | 100 | 2.0 | 4.3 |
| Pre-chlorination 9-100 LAP | - | 1.0 | 4.5 | 92 | 1.0 | 2.2 |
| | + | 1.0 | 6.4 | 136 | 6.0 | 9.4 |
| Post-chlorination 9-100 LAP | - | 1.0 | 6.3 | 129 | 2.0 | 3.2 |
| | - | 0.5 | 5.9 | 120 | 7.0 | 12 |
| | - | 0.25 | 6.3 | 129 | <1.0 | 1.6 |
| | - | 0.1 | 6.2 | 127 | 2.0 | 3.2 |
| | - | 0.02 | 6.2 | 127 | 5.0 | 8.1 |
| | + | 1.0 | 4.1 | 87 | 6.0 | 14.5 |
| | + | 0.5 | 4.1 | 87 | 5.0 | 12 |
| | + | 0 25 | 3.1 | 66 | 1.0 | 3.3 |
| | + | 0.1 | 4.6 | 98 | 6.0 | 13 |
| | + | 0.02 | 4.7 | 100 | 4.0 | 8.6 |

*100% Photolyzed

Table B-39

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 21

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.5 | 100 | 6.5 | 10 |
| | + | | 6.0 | 100 | 5.0 | 8.3 |
| Pre-chlorination 2,6-Dinitrotoluene | - | 0.000096 | 6.5 | 100 | 1.0 | 1.5 |
| | + | 0.000096 | 6.9 | 114 | 5.0 | 4.4 |
| Post-chlorination 2,6-Dinitrotoluene | - | 0.000096 | 10 | 157 | 5.0 | 4.9 |
| | - | 0.000048 | 7.1 | 109 | 6.0 | 8.5 |
| | - | 0.000024 | 7.9 | 121 | 6.0 | 7.6 |
| | - | 0.0000096 | 15 | 237 | 2.0 | 1.3 |
| | - | 0.0000019 | 7.2 | 111 | 1.0 | 1.4 |
| | + | 0.000096 | 8.9 | 147 | 5.0 | 5.6 |
| | + | 0.000048 | 8.2 | 135 | 4.0 | 4.9 |
| | + | 0.000024 | 6.3 | 105 | 4.0 | 6.3 |
| | + | 0.0000096 | 13 | 212 | 2.0 | 1.6 |
| | + | 0.0000019 | 6.0 | 99 | 2.0 | 3.4 |

Table B-40

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 23

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.7 | 100 | 7.0 | 10 |
| | + | | 6.1 | 100 | 10 | 16 |
| Pre-chlorination 2,6-Dinitrotoluene | - | 0.007 | 6.6 | 99 | 2.0 | 3.0 |
| | + | 0.007 | 5.3 | 87 | 3.0 | 5.7 |
| Post-chlorination 2,6-Dinitrotoluene | - | 0.007 | 6.7 | 100 | 6.0 | 9.0 |
| | - | 0.0035 | 5.3 | 79 | 5.0 | 9.4 |
| | - | 0.0018 | 5.2 | 78 | 1.0 | 1.9 |
| | - | 0.0007 | 5.6 | 84 | 5.0 | 8.9 |
| | - | 0.00014 | 5.8 | 87 | 3.0 | 5.2 |
| | + | 0.007 | 6.5 | 107 | 2.0 | 3.1 |
| | + | 0.0035 | 5.5 | 90 | 8.0 | 15 |
| | + | 0.0018 | 5.9 | 97 | 6.0 | 10 |
| | + | 0.0007 | 6.5 | 107 | 4.0 | 6.2 |
| | + | 0.00014 | 5.4 | 89 | 8.0 | 15 |

Table B-41

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 27

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 3.0 | 5.6 |
| | + | | 4.8 | 100 | 4.5 | 9.4 |
| Pre-ozonation 2,6-Dinitrotoluene | - | 0.0008 | 8.6 | 159 | 7.0 | 8.1 |
| | + | 0.0008 | 4.3 | 90 | 4.0 | 9.3 |
| Post-ozonation 2,6-Dinitrotoluene | - | 0.00035 | 4.2 | 78 | 1.0 | 2.4 |
| | - | 0.00018 | 4.3 | 80 | 5.0 | 12 |
| | - | 0.00009 | 4.0 | 74 | 5.0 | 13 |
| | - | 0.000035 | 4.5 | 83 | 2.0 | 4.4 |
| | - | 0.000007 | 4.0 | 74 | 11 | 28 |
| | + | 0.00035 | 4.1 | 85 | 6.0 | 15 |
| | + | 0.00018 | 4.4 | 92 | 3.0 | 6.8 |
| | + | 0.00009 | 4.7 | 98 | 5.0 | 11 |
| | + | 0.000035 | 4.1 | 85 | 2.0 | 4.9 |
| | + | 0.000007 | 4.0 | 83 | 8.0 | 20 |

Table B-42

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,6-DINITROTOLUENE

EXPERIMENT 34

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 13 | 22 |
| | + | | 5.2 | 100 | 5.8 | 11 |
| Pre-ozonation | - | 0.006 | 6.0 | 103 | 13 | 22 |
| 2,6-Dinitrotoluene | + | 0.006 | 5.9 | 113 | 9.0 | 15 |
| Post-ozonation | - | 0.0048 | 5.9 | 102 | 13 | 22 |
| 2,6-Dinitrotoluene | - | 0.0036 | 5.2 | 90 | 6.0 | 12 |
| | - | 0.0024 | 5.9 | 102 | 11 | 19 |
| | - | 0.0012 | 5.3 | 91 | 13 | 25 |
| | - | 0.00048 | 5.3 | 91 | 10 | 19 |
| | + | 0.0048 | 6.1 | 117 | 12 | 20 |
| | + | 0.0036 | 5.4 | 104 | 15 | 28 |
| | + | 0.0024 | 5.5 | 106 | 17 | 31 |
| | + | 0.0012 | 5.7 | 110 | 12 | 21 |
| | + | 0.00048 | 6.0 | 115 | 14 | 23 |

Table B-43

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4-DINITROTOLUENE

EXPERIMENT 1

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 5.0 | 8.6 |
| | + | | 5.5 | 100 | 3.5 | 6.3 |
| Pre-chlorination | - | 0.008 | 4.0 | 69 | 11 | 28 |
| 2,4-*Dinitrotoluene* | + | 0.008 | 5.2 | 93 | 7.0 | 13 |
| Post-chlorination | - | 0.008 | 5.1 | 87 | 8.0 | 16 |
| 2,4-Dinitrotoluene | - | 0.004 | 4.0 | 69 | 9.0 | 23 |
| | - | 0.002 | 4.8 | 83 | 5.0 | 10. |
| | - | 0.0008 | 4.2 | 72 | 2.0 | 4.8 |
| | - | 0.00016 | 3.4 | 58 | 1.0 | 3.0 |
| | + | 0.008 | 5.5 | 99 | 5.0 | 9.1 |
| | + | 0.004 | 5.4 | 97 | 3.0 | 5.6 |
| | + | 0.002 | 4.4 | 80 | 8.0 | 18 |
| | + | 0.0008 | 5.3 | 96 | 4.0 | 7.5 |
| | + | 0.00016 | 4.2 | 76 | 7.0 | 17 |

Table B-44

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4-DINITROTOLUENE

EXPERIMENT 28

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.8 | 100 | 5.5 | 11 |
| | + | | 5.9 | 100 | 5.5 | 9.3 |
| Pre-chlorination 2,4-Dinitrotoluene | - | 0.006 | 6.1 | 127 | 1.0 | 1.7 |
| | + | 0.006 | 6.8 | 115 | 2.0 | 2.9 |
| Post-chlorination 2,4-Dinitrotoluene | - | 0.006 | 3.6 | 75 | 3.0 | 8.3 |
| | - | 0.003 | 5.7 | 119 | 5.0 | 8.7 |
| | - | 0.0015 | 5.7 | 119 | 1.0 | 1.8 |
| | - | 0.0006 | 6.1 | 127 | 3.0 | 4.9 |
| | - | 0.00012 | 5.4 | 113 | 6.0 | 11 |
| | + | 0.006 | 4.7 | 80 | <1.0 | 2.1 |
| | + | 0.003 | 6.0 | 102 | 6.0 | 10 |
| | + | 0.0015 | 6.6 | 112 | 3.0 | 4.5 |
| | + | 0.0006 | 6.0 | 102 | 2.0 | 3.3 |
| | + | 0.00012 | 4.8 | 81 | <1.0 | 2.1 |

Table B-45

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
3,5-DINITROTOLUENE

EXPERIMENT 17

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 8.0 | 15 |
| | + | | 6.3 | 100 | 6.5 | 10 |
| Pre-chlorination 3,5-Dinitrotoluene | - | 0.005 | 4.7 | 88 | 8.0 | 17 |
| | + | 0.005 | 5.0 | 80 | 4.0 | 8.0 |
| Post-chlorination 3,5-Dinitrotoluene | - | 0.005 | 5.4 | 99 | 5.0 | 9.3 |
| | - | 0.0025 | 5.3 | 98 | 5.0 | 9.5 |
| | - | 0.0015 | 4.2 | 78 | 6.0 | 14 |
| | - | 0.0005 | 5.2 | 96 | 4.0 | 7.7 |
| | - | 0.0001 | 9.9 | 184 | 1.0 | 1.0 |
| | + | 0.005 | 5.6 | 89 | 1.0 | 1.8 |
| | + | 0.0025 | 5.4 | 86 | 7.0 | 13 |
| | + | 0.0015 | 4.7 | 75 | 5.0 | 11 |
| | + | 0.0005 | 5.7 | 91 | 3.0 | 5.3 |
| | + | 0.0001 | 5.1 | 81 | 4.0 | 7.9 |

Table B-46

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
3,5-DINITROTOLUENE

EXPERIMENT 18

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 5.0 | 8.6 |
| | + | | 5.5 | 100 | 3.5 | 6.3 |
| Pre-chlorination | - | 0.00067 | 4.7 | 82 | 6.0 | 13 |
| 3,5-Dinitrotoluene | + | 0.00067 | 4.8 | 87 | 3.0 | 6.3 |
| Post-chlorination | - | 0.00067 | 4.7 | 81 | 5.0 | 11 |
| 3,5-Dinitrotoluene | - | 0.00034 | 4.2 | 73 | 3.0 | 7.1 |
| | - | 0.00017 | 4.5 | 77 | 2.0 | 4.5 |
| | - | 0.000067 | 3.9 | 69 | 2.0 | 5.2 |
| | - | 0.000015 | 4.3 | 74 | 5.0 | 12 |
| | + | 0.00067 | 4.6 | 83 | 5.0 | 11 |
| | + | 0.00034 | 5.0 | 90 | 4.0 | 8.1 |
| | + | 0.00017 | 4.4 | 80 | 2.0 | 4.6 |
| | + | 0.000067 | 5.2 | 94 | 4.0 | 7.7 |
| | + | 0.000015 | 4.7 | 86 | 5.0 | 11 |

Table B-48

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 28

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.8 | 100 | 5.5 | 11 |
| | + | | 5.9 | 100 | 5.5 | 9.3 |
| Pre-chlorination 2,4,6-Trinitrotoluene | - | 0.003 | 6.3 | 131 | 8.0 | 13 |
| | + | 0.003 | 5.1 | 86 | 8.3 | 16 |
| Post-chlorination 2,4,6-Trinitrotoluene | - | 0.003 | 6.5 | 135 | 10 | 15 |
| | - | 0.0015 | 6.1 | 127 | 5.0 | 8.2 |
| | - | 0.008 | 4.9 | 102 | 4.0 | 8.2 |
| | - | 0.0003 | 4.9 | 102 | 4.0 | 8.1 |
| | - | 0.00006 | 5.2 | 108 | 6.0 | 11 |
| | + | 0.003 | 5.4 | 92 | 1.0 | 1.9 |
| | + | 0.0015 | 5.1 | 86 | 3.0 | 5.9 |
| | + | 0.008 | 4.3 | 73 | 3.0 | 5.7 |
| | + | 0.0003 | 5.3 | 90 | 3.0 | 5.7 |
| | + | 0.00006 | 6.2 | 105 | 2.0 | 3.2 |

Table B-47

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 18

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.8 | 100 | 5.0 | 8.6 |
| | + | | 5.5 | 100 | 3.5 | 6.3 |
| Pre-chlorination 2,4,6-Trinitrotoluene | - | 0.006 | 5.4 | 93 | 3.0 | 5.6 |
| | + | 0.006 | 4.9 | 88 | 1.0 | 2.1 |
| Post-chlorination 2,4,6-Trinitrotoluene | - | 0.006 | 5.5 | 94 | 5.0 | 9.1 |
| | - | 0.003 | 4.8 | 83 | 6.0 | 13 |
| | - | 0.0015 | 4.9 | 84 | 1.0 | 2.1 |
| | - | 0.0006 | 5.0 | 87 | 5.0 | 10.0 |
| | - | 0.00012 | 4.8 | 83 | 6.0 | 13 |
| | + | 0.006 | 6.4 | 116 | 3.0 | 4.7 |
| | + | 0.003 | 4.7 | 85 | 4.0 | 8.5 |
| | + | 0.0015 | 4.0 | 72 | 5.0 | 13 |
| | + | 0.0006 | 4.0 | 73 | 3.0 | 7.4 |
| | + | 0.00012 | 5.0 | 91 | 3.0 | 6.0 |

Table B-49

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 27

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 3.0 | 5.6 |
| | + | | 4.8 | 100 | 4.5 | 9.4 |
| Pre-ozonation | - | 0.0012 | 5.3 | 98 | 1.0 | 1.9 |
| 2,4,6-Trinitrotoluene | + | 0.0012 | 4.5 | 94 | 4.0 | 8.9 |
| Post-ozonation | - | 0.001 | 5.5 | 102 | 4.0 | 7.3 |
| 2,4,6-Trinitrotoluene | - | 0.0005 | 5.7 | 106 | 3.0 | 5.3 |
| | - | 0.00025 | 4.2 | 78 | 7.0 | 17 |
| | - | 0.0001 | 4.3 | 80 | 1.0 | 2.3 |
| | - | 0.00002 | 4.8 | 89 | 2.0 | 4.2 |
| | + | 0.001 | 5.2 | 108 | 3.0 | 5.8 |
| | + | 0.0005 | 5.2 | 108 | 5.0 | 9.6 |
| | + | 0.00025 | 5.0 | 104 | 9.0 | 18 |
| | + | 0.0001 | 4.8 | 100 | 8.0 | 17 |
| | + | 0.00002 | 4.4 | 92 | 6.0 | 14 |

Table B-50

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROTOLUENE

EXPERIMENT 36

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 7.6 | 100 | 5.0 | 6.6 |
| | + | | 8.8 | 100 | 7.0 | 8.0 |
| Pre-ozonation 2,4,6-Trinitrotoluene | - | 0.0015 | 11 | 145 | 4.0 | 3.6 |
| | + | 0.0015 | 9.9 | 113 | 6.3 | 6.4 |
| Post-ozonation 2,4,6-Trinitrotoluene | - | 0.0013 | 9.4 | 124 | 2.0 | 2.1 |
| | - | 0.0009 | 9.0 | 118 | 3.0 | 3.3 |
| | - | 0.0006 | 9.7 | 128 | 8.0 | 8.2 |
| | - | 0.0003 | 9.5 | 125 | 3.0 | 3.2 |
| | - | 0.00013 | 9.7 | 128 | 9.0 | 9.3 |
| | + | 0.0013 | 8.2 | 93 | 7.5 | 9.1 |
| | + | 0.0009 | 9.3 | 106 | 7.0 | 7.5 |
| | + | 0.0006 | 8.3 | 94 | 4.0 | 4.8 |
| | + | 0.0003 | 9.9 | 113 | 3.8 | 3.8 |
| | + | 0.00013 | 10 | 114 | 4.0 | 4.0 |

Table B-51

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 17

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 8.0 | 15 |
| | + | | 6.3 | 100 | 6.5 | 10 |
| Pre-chlorination 2,4,6-Trinitroresorcinol | - | 0.03 | 6.0 | 111 | 8.0 | 13 |
| | + | 0.03 | 5.2 | 82 | 5.0 | 9.7 |
| Post-chlorination 2,4,6-Trinitroresorcinol | - | 0.028 | 5.8 | 107 | 3.0 | 5.2 |
| | - | 0.014 | 5.3 | 98 | 5.0 | 9.5 |
| | - | 0.007 | 4.9 | 91 | 4.0 | 8.1 |
| | - | 0.0028 | 5.2 | 96 | 5.0 | 9.7 |
| | - | 0.00056 | 5.6 | 103 | 5.0 | 9.0 |
| | + | 0.028 | 5.8 | 92 | 5.0 | 8.7 |
| | + | 0.014 | 5.9 | 93 | 5.0 | 8.5 |
| | + | 0.007 | 5.1 | 82 | 5.0 | 9.8 |
| | + | 0.0028 | 6.0 | 96 | 1.0 | 1.7 |
| | + | 0.00056 | 5.0 | 79 | 2.0 | 4.0 |

Table B-52

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 28

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.8 | 100 | 5.5 | 11 |
| | + | | 5.9 | 100 | 5.5 | 9.3 |
| Pre-chlorination | - | 0.02 | 5.8 | 121 | 6.0 | 10 |
| 2,4,6-Trinitroresorcinol | + | 0.02 | 6.4 | 108 | 4.0 | 6.3 |
| Post-chlorination | - | 0.19 | 6.3 | 131 | 2.0 | 3.2 |
| 2,4,6-Trinitroresorcinol | - | 0.10 | 5.7 | 119 | 4.0 | 7.0 |
| | - | 0.005 | 7.3 | 152 | 2.0 | 2.7 |
| | - | 0.0019 | 6.8 | 142 | 7.0 | 10 |
| | - | 0.0004 | 7.8 | 163 | 4.0 | 5.1 |
| | + | 0.19 | 6.2 | 105 | 9.0 | 15 |
| | + | 0.10 | 6.3 | 107 | 4.0 | 6.3 |
| | + | 0.005 | 8.2 | 139 | 4.0 | 4.9 |
| | + | 0.0019 | 6.7 | 114 | 7.0 | 10 |
| | + | 0.0004 | 7.9 | 134 | 3.0 | 3.8 |

Table B-53

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 28

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 4.4 | 100 | 3.5 | 8.0 |
| | + | | 4.6 | 100 | 3.5 | 7.6 |
| Pre-ozonation | - | 0.033 | 5.3 | 120 | 8.0 | 15 |
| 2,4,6-Trinitroresorcinol | + | 0.033 | 4.1 | 89 | 4.0 | 9.8 |
| Post-ozonation | - | 0.029 | 5.4 | 123 | 4.0 | 7.4 |
| 2,4,6-Trinitroresorcinol | - | 0.015 | 5.4 | 123 | 8.0 | 15 |
| | - | 0.007 | 7.6 | 173 | 4.0 | 5.3 |
| | - | 0.0029 | 4.6 | 105 | 3.0 | 6.5 |
| | - | 0.0006 | 5.0 | 114 | 5.0 | 10 |
| | + | 0.029 | 5.4 | 117 | 7.0 | 13 |
| | + | 0.015 | 8.7 | 189 | 5.0 | 5.7 |
| | + | 0.007 | 7.5 | 163 | 3.0 | 4.0 |
| | + | 0.0029 | 5.6 | 122 | 5.0 | 8.9 |
| | + | 0.0006 | 4.7 | 102 | 6.0 | 13 |

Table B-54

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITRORESORCINOL

EXPERIMENT 35

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 6.6 | 100 | 3.8 | 5.8 |
| | + | | 8.0 | 100 | 3.7 | 4.6 |
| Pre-ozonation 2,4,6-Trinitroresorcinol | - | 0.012 | 7.5 | 114 | 5.0 | 6.7 |
| | + | 0.012 | 7.6 | 95 | 9.0 | 12 |
| Post-ozonation 2,4,6-Trinitroresorcinol | - | 0.010 | 7.0 | 106 | 9.0 | 13 |
| | - | 0.0075 | 7.4 | 112 | 6.0 | 8.1 |
| | - | 0.0050 | 7.1 | 108 | 6.0 | 8.5 |
| | - | 0.0025 | 6.2 | 94 | 6.3 | 10 |
| | - | 0.0010 | 6.1 | 92 | 9.0 | 15 |
| | + | 0.010 | 5.4 | 68 | 3.0 | 5.6 |
| | + | 0.0075 | 6.4 | 80 | 3.0 | 4.7 |
| | + | 0.0050 | 5.7 | 71 | 6.0 | 11 |
| | + | 0.0025 | 6.1 | 76 | 6.0 | 9.8 |
| | + | 0.0010 | 6.5 | 81 | 5.0 | 7.7 |

Table B-55

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

| Compound | Metabolic Activation | | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 12 | 100 | 7.5 | 6.3 |
| | + | | 9.1 | 100 | 8.5 | 9.3 |
| Pre-chlorination 2,4,6-Trinitrobenzonitrile | - | 1 | 6.3 | 53 | 5.0 | 7.9 |
| | + | 1 | 5.8 | 64 | 4.0 | 6.9 |
| Post-chlorination 2,4,6-Trinitrobenzonitrile | - | 1 | 5.7 | 48 | 3.0 | 5.3 |
| | - | 0.5 | 5.5 | 46 | 5.0 | 9.1 |
| | - | 0.25 | 7.3 | 61 | 3.0 | 4.1 |
| | - | 0.1 | 20 | 167 | 8.0 | 4.0 |
| | - | 0.02 | 32 | 267 | 3.0 | 0.9 |
| | + | 1 | 5.3 | 58 | 7.0 | 13 |
| | + | 0.5 | 6.2 | 68 | 9.0 | 15 |
| | + | 0.25 | 6.3 | 69 | 8.0 | 13 |
| | + | 0.1 | 12 | 132 | 2.0 | 1.5 |
| | + | 0.02 | 16 | 176 | 8.0 | 5.0 |

*Compound 100% decomposition to picric acid and other compounds.

Table B-56

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 31

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 7.0 | 100 | 5.0 | 7.1 |
| | + | | 7.0 | 100 | 5.5 | 7.9 |
| Pre-chlorination 2,4,6-Trinitrobenzonitrile | - | 1 | 7.7 | 110 | 3.0 | 3.9 |
| | + | 1 | 7.5 | 107 | 9.0 | 12 |
| Post-chlorination 2,4,6-Trinitrobenzonitrile | - | 1 | 6.6 | 94 | 9.0 | 14 |
| | - | 0.75 | 6.4 | 91 | 4.0 | 6.3 |
| | - | 0.5 | 7.8 | 111 | 5.0 | 6.4 |
| | - | 0.25 | 7.2 | 103 | 4.0 | 5.6 |
| | - | 0.1 | 5.7 | 81 | 3.0 | 5.3 |
| | + | 1 | 6.5 | 93 | 4.0 | 6.2 |
| | + | 0.75 | 6.8 | 97 | 2.0 | 2.9 |
| | + | 0.5 | 6.1 | 87 | 6.0 | 9.8 |
| | + | 0.25 | 6.5 | 93 | 9.0 | 14 |
| | + | 0.1 | 5.3 | 76 | 5.0 | 9.4 |

*Compound 100% decomposition into picric acid and other compounds.

Table B-57

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 26

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 12 | 100 | 7.5 | 6.3 |
| | + | | 9.1 | 100 | 8.5 | 9.3 |
| Pre-ozonation 2,4,6-Trinitrobenzonitrile | - | 1 | 6.3 | 53 | 7.0 | 11 |
| | + | 1 | 5.3 | 58 | 2.0 | 3.8 |
| Post-ozonation 2,4,6-Trinitrobenzonitrile | - | 1 | 6.7 | 56 | 9.0 | 13 |
| | - | 0.5 | 6.9 | 58 | 3.8 | 5.5 |
| | - | 0.25 | 6.2 | 52 | 4.0 | 6.5 |
| | - | 0.1 | 7.8 | 65 | 2.0 | 2.6 |
| | - | 0.02 | 6.5 | 54 | 5.0 | 7.7 |
| | + | 1 | 5.2 | 57 | 7.0 | 13 |
| | + | 0.5 | 7.0 | 77 | 1.3 | 1.9 |
| | + | 0.25 | 6.3 | 69 | 6.0 | 9.5 |
| | + | 0.1 | 7.7 | 85 | 4.0 | 5.2 |
| | + | 0.02 | 6.7 | 74 | 7.0 | 10 |

*Compound 100% decomposition to picric acid and other compounds.

Table B-58

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZONITRILE*

EXPERIMENT 36

| Compound | Metabolic Activation | Milliliters of Solution Added | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|
| Negative control | - | | 7.6 | 100 | 5.0 | 6.6 |
| | + | | 8.8 | 100 | 7.0 | 8.0 |
| Pre-ozonation 2,4,6-Trinitrobenzonitrile | - | 1 | 7.4 | 97 | 4.0 | 5.4 |
| | + | 1 | 7.9 | 90 | 3.0 | 3.8 |
| Post-ozonation 2,4,6-Trinitrobenzonitrile | - | 1 | 9.8 | 129 | 7.0 | 7.1 |
| | - | 0.75 | 9.9 | 130 | 1.0 | 1.0 |
| | - | 0.5 | 9.6 | 126 | 3.0 | 3.1 |
| | - | 0.25 | 8.7 | 114 | 3.0 | 3.4 |
| | - | 0.1 | 9.5 | 125 | 3.0 | 3.2 |
| | + | 1 | 7.8 | 89 | 7.0 | 9.0 |
| | + | 0.75 | 9.8 | 111 | 6.0 | 6.1 |
| | + | 0.5 | 9.4 | 107 | 5.0 | 5.3 |
| | + | 0.25 | 8.1 | 92 | 4.0 | 4.9 |
| | + | 0.1 | 7.2 | 82 | 1.7 | 2.4 |

* 100% Decomposition to picric acid and other compounds.

Table B-59

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 3.0 | 5.6 |
| | + | | 4.8 | 100 | 4.5 | 9.4 |
| Pre-chlorination | - | 0.0004 | 3.8 | 70 | 3.0 | 7.9 |
| 2,4,6-Trinitrobenzaldehyde | + | 0.0004 | 3.6 | 75 | 3.0 | 8.3 |
| Post-chlorination | - | 0.0004 | 2.6 | 48 | 2.0 | 7.7 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.0002 | 3.2 | 59 | 2.0 | 6.3 |
| | - | 0.0001 | 3.3 | 61 | 3.0 | 9.1 |
| | - | 0.00004 | 3.5 | 65 | 3.0 | 8.6 |
| | - | 0.000008 | 3.7 | 69 | 6.0 | 16 |
| | + | 0.0004 | 5.1 | 106 | 3.0 | 5.9 |
| | + | 0.0002 | 3.3 | 69 | 3.0 | 9.1 |
| | + | 0.0001 | 3.4 | 71 | 3.0 | 8.8 |
| | + | 0.00004 | 3.2 | 67 | 2.0 | 6.3 |
| | + | 0.000008 | 3.6 | 75 | 2.0 | 5.6 |

Table B-60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 31

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-5}$) | Per $10^5$ Survivors |
| Negative control | - | | 7.0 | 100 | 5.0 | 7.1 |
| | + | | 7.0 | 100 | 5.5 | 7.9 |
| Pre-chlorination | - | 0.002 | C* | C | C | C |
| 2,4,6-Trinitrobenzaldehyde | + | 0.002 | 7.3 | 104 | 5.0 | 6.8 |
| Post-chlorination | - | 0.0015 | 12 | 171 | 6.0 | 5.0 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.0011 | --† | -- | -- | -- |
| | - | 0.0007 | 5.9 | 84 | 3.0 | 5.1 |
| | - | 0.00035 | 5.9 | 84 | 3.0 | 5.1 |
| | - | 0.00015 | C* | C* | C* | C* |
| | + | 0.0015 | 9.5 | 136 | 4.0 | 4.2 |
| | + | 0.0011 | -- | -- | -- | -- |
| | + | 0.0007 | 6.8 | 97 | 7.0 | 10 |
| | + | 0.00035 | 5.9 | 84 | 4.0 | 6.8 |
| | + | 0.00015 | 10 | 143 | 3.0 | 3.0 |

*C, Contaminated

† Dilution error.

Table B-61

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 27

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 5.4 | 100 | 3.0 | 5.6 |
| | + | | 4.8 | 100 | 4.5 | 9.4 |
| Pre-ozonation | - | 0.0005 | 2.9 | 54 | 5.0 | 17 |
| 2,4,6-Trinitrobenzaldehyde | + | 0.0005 | 3.3 | 69 | 3.0 | 9.1 |
| Post-ozonation | - | 0.00034 | 3.0 | 56 | 4.0 | 13 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.00017 | 3.9 | 72 | 5.0 | 13 |
| | - | 0.00009 | 3.9 | 72 | 4.0 | 10 |
| | - | 0.000034 | 3.5 | 65 | 2.0 | 5.7 |
| | - | 0.000007 | 4.7 | 87 | 9.0 | 19 |
| | + | 0.00034 | 5.2 | 108 | 4.0 | 7.7 |
| | + | 0.00017 | 3.6 | 75 | 9.0 | 25 |
| | + | 0.00009 | 2.7 | 56 | 6.0 | 22 |
| | + | 0.000034 | 3.3 | 69 | 2.0 | 6.1 |
| | + | 0.000007 | 3.5 | 73 | 6.0 | 17 |

Table B-62

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE
2,4,6-TRINITROBENZALDEHYDE

EXPERIMENT 36

| Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|
| | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| Negative control | - | | 7.6 | 100 | 5.0 | 6.6 |
| | + | | 8.8 | 100 | 7.0 | 8.0 |
| Pre-ozonation | - | 0.0028 | 9.5 | 125 | 6.0 | 6.3 |
| 2,4,6-Trinitrobenzaldehyde | + | 0.0028 | 8.8 | 116 | 4.0 | 4.5 |
| Post-ozonation | - | 0.0025 | 9.3 | 122 | 1.0 | 1.1 |
| 2,4,6-Trinitrobenzaldehyde | - | 0.0018 | 7.4 | 97 | 6.0 | 8.1 |
| | - | 0.0012 | 7.7 | 101 | 13 | 17 |
| | - | 0.0006 | 9.3 | 122 | 4.0 | 4.3 |
| | - | 0.00025 | 8.5 | 112 | 5.0 | 5.9 |
| | + | 0.0025 | 8.2 | 93 | 9.0 | 11 |
| | + | 0.0018 | 8.0 | 91 | 5.0 | 6.3 |
| | + | 0.0012 | 7.9 | 90 | 6.0 | 7.6 |
| | + | 0.0006 | 8.7 | 99 | 6.0 | 6.9 |
| | + | 0.00025 | 8.8 | 100 | 4.0 | 4.5 |

Table B-63

SACCHAROMYCES CEREVISIAE EXPERIMENTAL POSITIVE CONTROLS

| Exp. No. | Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|---|
| | | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| 1 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.0 | 75 | 851 | 1702 |
| | | + | 0.5 | 5.6 | 80 | 934 | 1668 |
| 2 | 1,2,3,4-Diepoxybutane | - | 0.5 | 8.2 | 178 | 590 | 720 |
| | | + | 0.5 | 2.6 | 27 | 630 | 2471 |
| 3 | 1,2,3,4-Diepoxybutane | - | 0.5 | 1.1 | 19 | 580 | 5272 |
| | | + | 0.5 | 0.4 | 8 | 176 | 4190 |
| 4 | 1,2,3,4-Diepoxybutane | - | 0.5 | 3.1 | 103 | 865 | 2808 |
| | | + | 0.5 | T* | T | T | T |
| 5 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.2 | 55 | 213 | 504 |
| | | + | 0.5 | T | T | T | T |
| 6 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.2 | 84 | 1013 | 2401 |
| | | + | 0.5 | 6.4 | 112 | 988 | 1542 |
| 7 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.3 | 83 | 1088 | 2547 |
| | | + | 0.5 | 4.5 | 92 | 1087 | 2443 |
| 8 | 1,2,3,4-Diepoxybutane | - | 0.5 | 7.2 | 91 | 1282 | 1788 |
| | | + | 0.5 | 7.3 | 54 | 1680 | 2301 |
| 9 | 1,2,3,4-Diepoxybutane | - | 0.5 | 6.4 | 85 | 865 | 1352 |
| | | + | 0.5 | 14.7 | 201 | 938 | 638 |
| 10 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.6 | 100 | 1253 | 2250 |
| | | + | 0.5 | 5.2 | 96 | 1033 | 1979 |

*T, Toxic

Table B-63 (continued)

| Exp. No. | Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|---|
| | | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| 11 | 1,2,3,4-Diepoxybutane | - | 0.5 | 3.1 | 53 | 785 | 2532 |
| | | + | 0.5 | 4.8 | 87 | 833 | 1753 |
| 12 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.5 | 71 | 1425 | 3181 |
| | | + | 0.5 | 4.9 | 83 | 1228 | 2495 |
| 13 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.7 | 89 | 1108 | 2346 |
| | | + | 0.5 | T | T | T | T |
| 14 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.4 | 85 | 1420 | 3215 |
| | | + | 0.5 | 6.2 | 105 | 1368 | 2218 |
| 15 | 1,2,3,4-Diepoxybutane | - | 0.5 | 0.8 | 5 | 390 | 5200 |
| | | + | 0.5 | 6.0 | 34 | 730 | 1217 |
| 16 | 1,2,3,4-Diepoxybutane | - | 0.5 | 2.8 | 44 | 978 | 3516 |
| | | + | 0.5 | 2.9 | 49 | 738 | 2569 |
| 17 | 1,2,3,4-Diepoxybutane | - | 0.5 | 3.4 | 63 | 715 | 2115 |
| | | + | 0.5 | 6.5 | 103 | 1590 | 2458 |
| 18 | 1,2,3,4-Diepoxybutane | - | 0.5 | 2.7 | 47 | 1128 | 4176 |
| | | + | 0.5 | 1.8 | 33 | 660 | 3667 |
| 19 | 1,2,3,4-Diepoxybutane | - | 0.5 | T | T | T | T |
| | | + | 0.5 | 5.6 | 85 | 1153 | 2058 |
| 20 | 1,2,3,4-Diepoxybutane | - | 0.5 | 2.9 | 43 | 1023 | 3526 |
| | | + | 0.5 | 5.0 | 76 | 1318 | 2635 |
| 21 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.9 | 91 | 1545 | 2619 |
| | | + | 0.5 | 6.9 | 115 | 1413 | 2047 |

Table B-63 (continued)

| Exp. No. | Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors | | Mitotic Recombinants | |
|---|---|---|---|---|---|---|---|
| | | | | Cells per ml ($\times 10^{-7}$) | Percent | Per ml ($\times 10^{-3}$) | Per $10^5$ Survivors |
| 22 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.4 | 67 | 1278 | 2923 |
| | | + | 0.5 | 5.9 | 97 | 1663 | 2834 |
| 23 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.9 | 88 | 1148 | 1945 |
| | | + | 0.5 | 5.6 | 92 | 1295 | 2313 |
| 24 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.5 | 76 | 1468 | 3261 |
| | | + | 0.5 | 4.5 | 85 | 1515 | 3368 |
| 25 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.8 | 83 | 1498 | 3120 |
| | | + | 0.5 | 4.2 | 84 | 1325 | 3155 |
| 26 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.5 | 44 | 995 | 1809 |
| | | + | 0.5 | 3.0 | 33 | 1020 | 3400 |
| 27 | 1,2,3,4-Diepoxybutane | - | 0.5 | 4.4 | 81 | 1183 | 2688 |
| | | + | 0.5 | 3.7 | 77 | 1048 | 2831 |
| 28 | 1,2,3,4-Diepoxybutane | - | 0.5 | T | T | T | T |
| | | + | 0.5 | 4.7 | 80 | 1263 | 2686 |
| 29 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.3 | 100 | 1443 | 2722 |
| | | + | 0.5 | 5.5 | 93 | 1253 | 2277 |
| 30 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.3 | 87 | 1705 | 3217 |
| | | + | 0.5 | 5.7 | 83 | 1366 | 2398 |
| 31 | 1,2,3,4-Diepoxybutane | - | 0.5 | 6.2 | 89 | 1363 | 2198 |
| | | + | 0.5 | 6.4 | 91 | 1350 | 2109 |
| 32 | 1,2,3,4-Diepoxybutane | - | 0.5 | 6.4 | 128 | 1085 | 1695 |
| | | + | 0.5 | 5.7 | 100 | 1393 | 2443 |

Table B-63 (continued)

| Exp. No. | Compound | Metabolic Activation | Percent Concentration (w/v or v/v) | Survivors: Cells per ml ($\times 10^{-7}$) | Survivors: Percent | Mitotic Recombinants: Per ml ($\times 10^{-3}$) | Mitotic Recombinants: Per $10^5$ Survivors |
|---|---|---|---|---|---|---|---|
| 33 | 1,2,3,4-Diepoxybutane | - | 0.5 | 3.3 | 72 | 835 | 2511 |
| | | + | 0.5 | 3.2 | 67 | 580 | 1803 |
| 34 | 1,2,3,4-Diepoxybutane | - | 0.5 | 3.9 | 67 | 988 | 2543 |
| | | + | 0.5 | 3.5 | 67 | 818 | 2319 |
| 35 | 1,2,3,4-Diepoxybutane | - | 0.5 | 6.4 | 97 | 785 | 1227 |
| | | + | 0.5 | 4.9 | 61 | 734 | 1498 |
| 36 | 1,2,3,4-Diepoxybutane | - | 0.5 | 7.3 | 96 | 1088 | 1493 |
| | | + | 0.5 | 5.2 | 59 | 1222 | 2342 |
| 37 | Not tested | | | | | | |
| 38 | Not tested | | | | | | |
| 39 | Not tested | | | | | | |
| 40 | Not tested | | | | | | |
| 41 | 1,2,3,4-Diepoxybutane | - | 0.5 | 0.5 | 14 | 150 | 3000 |
| | | + | 0.5 | 2.3 | 18 | 910 | 3957 |
| 42 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.0 | 74 | 928 | 1855 |
| | | + | 0.5 | 5.6 | 75 | 925 | 1652 |
| 43 | 1,2,3,4-Diepoxybutane | - | 0.5 | 5.4 | 95 | 1213 | 2246 |
| | | + | 0.5 | 4.9 | 92 | 1065 | 2173 |
| 44 | 1,2,3,4-Diepoxybutane | - | 0.5 | 2.8 | 57 | 995 | 3596 |
| | | + | 0.5 | 3.8 | 81 | 63 | 165 |
| 45 | Not tested | | | | | | |

APPENDIX C

Abstract of Poster Presentation at
Sixteenth Annual Meeting, Society of
Toxicology, Toronto, Canada, March
27-30, 1977

MUNITIONS WASTEWATER TREATMENTS: DOES CHLORINATION OR OZONATION OF INDIVIDUAL COMPONENTS PRODUCE MICROBIAL MUTAGENS? V. F. Simmon, S. L. Eckford, A. F. Griffin, R. Spanggord, and G. W. Newell, SRI International, Menlo Park, California*

*Abstract No. 157 in: Toxicology and Applied Pharmacology, 41, 197 (1977).

## Abstract

A number of compounds present in wastewater from munitions plants were examined before and after ozonation or chlorination to determine whether any were mutagenic before treatment and whether such activity was affected by the treatment. Several photolytic as well as metabolic products of trinitrotoluene (TNT) also were examined for mutagenic activity. Test materials included TNT, TNT production wastewater and individual components of TNT wastewater (1,3-dinitrobenzene; 2,4-dinitrotoluene; 3,5-dinitrotoluene; 1,3,5-trinitrohexahydro-1,3,5-trizine (RDX); 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX); components of photolysed TNT; pentaerythritol tetranitrate (PETN); and trinitroresorcinol. The *in vitro* mutagenic assays used were the Ames *Salmonella*/microsome assay (strains TA1535, TA1537, TA1538, TA98, and TA100) and mitotic recombination in the yeast, *Saccharomyces cerevisiae* D3. A metabolic activation system using the post-mitochondrial supernatant fraction of liver from rats pretreated with Aroclor 1254 was included in each assay procedure. Materials found to be mutagenic prior to and after treatment were trinitrobenzene, trinitrobenzaldehyde, trinitrobenzonitrile, and 50% and 100% photolysed TNT wastewater. Neither ozonation nor chlorination significanlty altered the mutagenic activity of the materials tested. (Supported by the U.S. Army Medical Research and Development Command, under Contract No. DAMD 17-76-C-6013.)

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